

A COMPARISON OF NEODYMIUM: YTTRIUM, ALUMINUM,  
GARNET LASER EFFECTS BETWEEN PRIMARY AND  
PERMANENT ENAMEL OF DISASSOCIATED TEETH

By

Nora Najeeb Tleel

Submitted to the Graduate Faculty of the School of  
Dentistry in partial fulfillment of the requirements  
for the degree of Master of Science in Dentistry,  
Indiana University School of Dentistry, 1995.

Thesis accepted by the faculty of the Department of Pediatric Dentistry, Indiana University School of Dentistry, in partial fulfillment of the requirements for the degree of Master of Science in Dentistry.

---

David R. Avery

---

Brian J. Sanders

---

Bruce R. Schemehorn

---

Gerald C. Pruesz

---

Jeffrey A. Dean

Chair of the Committee

Date



DEDICATION

I dedicate this thesis to my parents, Lina and Najeeb Tleel, and my friends, Alane and Fredrick Klem. Without their love, support and encouragement, this thesis would not have been completed.

## ACKNOWLEDGMENTS



I would like to thank the members of my graduate committee, Dr. Jeffrey Dean, Mr. Bruce Schemehorn, Dr. David Avery, Dr. Brian Sanders and Dr. Gerald Preusz for their continuous support and their generous help, time and input.

Sincere appreciation also goes to the Oral Health research facility staff for all their help in many of the laboratory testing procedures and the use of their facilities. Thanks also goes to Mr. Tim Noblitt and the staff for all their time spent with me in the use of the Confocal and the Scanning Electron microscopes.

I am grateful for the assistance of Dr. Barry Katz and George Eckert for providing the statistical analysis of the data. Credit is given for the high quality of the illustrated figures and photos created by Mr. Mark Dirlam, Mr. Mike Halloran, and Ms. Alana Barra. My appreciation is also extended to Ms. Julie LeHunt for all her assistance in typing and editing this thesis.

I would also like to extend my appreciation to the Riley Hospital dental clinic staff for all their support and encouragement.

Most important, I would like to express my love and appreciation to my family, Najeeb, Lina, Ibrahim and Anita, and to my good friends Alane and Fred Klem. They have supported me in all my decisions, even when it meant sacrifices for them. Without their patience, understanding, support and encouragement this research project would have never been completed and presented in its final form.



## TABLE OF CONTENTS

Introduction .....	1
Review of Literature .....	4
Methods and Materials .....	2 2
Results .....	3 0
Tables and Figures .....	3 8
Discussion .....	7 4
Summary and Conclusions .....	8 7
References .....	9 1
Appendixes .....	9 7
Abstract. ....	1 0 9
Curriculum Vitae	

LIST OF ILLUSTRATIONS



TABLE I	Statistical analysis of calcium and phosphorus dissassociation for lased (L), non-lased (NL), permanent (Per) and primary (Pri) enamel.....	39
TABLE IA	The individual readings for calcium and phosphorus dissassociation for lased (L) and non-lased (NL) permanent (Per) teeth.....	40
TABLE IB	The individual readings for calcium and phosphorus dissassociation for lased (L) and non-lased (NL) primary (Pri) teeth.....	41
TABLE II	Statistical analysis of the Vicker's Hardness Test of all chips [permanent (Per), primary (Pri), lased (L) and non-lased (NL)]......	42
TABLE IIA	All four individual readings of Vicker's Hardness Test reading #1 — control lased (L) and non-lased (NL) permanent (Per) chips.....	43
TABLE IIB	All four individual readings of Vicker's Hardness Test reading #1 — control primary (Pri) chips.....	44
TABLE IIC	All four readings of Vicker's Hardness Test reading #2 after lasing of permanent (Per) chips.....	45
TABLE IID	All four readings of Vicker's Hardness Test reading #2 after lasing of primary (Pri) chips.....	46
TABLE IIE	All four readings of Vicker's Hardness Test reading #3 after acid etching of permanent (Per) chips.....	47



TABLE IIF	All four readings of Vicker's Hardness Test reading #3 after acid etching of primary (Pri) chips.....	48
TABLE III	Statistical analysis of the Vicker's Hardness Tests of reading #2 minus base line, reading #3 minus base line, and reading #3 minus reading #2.....	49
TABLE IIIA	The individual calculations of each chip for reading #2 minus base line, reading #3 minus base line and reading #3 minus reading #2.....	50
TABLE IV	Statistical analysis of the confocal observations on all lased (L), non lased (NL) permanent (Per) and primary (Pri) hips.....	51
TABLE IVA	The individual readings of all areas, left and right, on all lased (L) and non-lased (NL) permanent (Per) and primary (Pri) chips.....	52
FIGURE 1	Endo Technic laser - 35 .....	53
FIGURE 2	Mounted 3 mm enamel chip on a lucite rod.....	54
FIGURE 3	Contact handpiece of the Nd:YAG laser.....	55
FIGURE 4	Diagram illustrating the four different areas on the enamel chip for the SEM study.....	56
FIGURE 5	3 mm round diamond cutting cylinder, a part of the drill press machine.....	57
FIGURE 6	Drill press machine used to produce 3 mm chips of tooth structure.....	57

FIGURE 7	Set up for grinding and polishing the enamel chips.....	58
FIGURE 8	Set up for the Vicker's Hardness Test.....	59
FIGURE 9	Placing the 200 g load for the hardness test.....	59
FIGURE 10	Applying the 200 g load on the enamel chip.....	60
FIGURE 11	Diagram indicating the areas where the hardness tests were done and computed.....	61
FIGURE 12	Diagram showing the cross section of the enamel chip and the lucite rod.....	62
FIGURE 13	SEM: Primary smooth surface, non-lased/non etched.....	63
FIGURE 14	SEM: Primary smooth surface, non-lased and acid etched. Enamel prisms with smooth surface indicated.....	63
FIGURE 15	SEM: Permanent smooth surface, lased and acid etched. Debris and scratches seen.....	64
FIGURE 16	SEM: Permanent smooth surface, lased and non etched. Smooth surface with debris and scratches noted.....	64
FIGURE 17	SEM: Primary smooth surface, specimen #4. Scratches were seen in all four areas.....	65
FIGURE 18	SEM: Permanent pit and fissure, lased/acid etched, cracking.....	66



FIGURE 19	SEM: Permanent, pit and fissure, non-lased and etched. Enamel prisms.....	66
FIGURE 20	SEM: Permanent, pit and fissure, lased and non etched, cratering.....	67
FIGURE 21	SEM: Permanent, pit and fissure, lased and acid etched, white bubbles and cobblestone appearance.....	67
FIGURE 22	SEM: Primary, pit and fissure, lased and non etched. Rough surface.....	68
FIGURE 23	SEM: Primary, pit and fissure, non-lased and non etched, ridges.....	68
FIGURE 24	SEM: Primary, pit and fissure, lased and non etched, ravines.....	69
FIGURE 25	SEM: Primary, pit and fissure, lased and acid etched, smooth surface.....	69
FIGURE 26	SEM: Permanent, pit and fissure, lased and non etched, cratering and pitting.....	70
FIGURE 27	SEM: Permanent, pit and fissure, lased and etched, cratering and pitting.....	70
FIGURE 28	Confocal, primary lased, 250 micron area of demineralized enamel of 6600.35 square microns.....	71
FIGURE 29	Confocal, primary non-lased 250 micron area of demineralized enamel of 8124.40 square microns.....	71

FIGURE 30	Confocal, permanent lased, 250 micron area of demineralized enamel of 6253.50 square microns.....	72
FIGURE 31	Confocal, permanent non-lased 250 micron area of demineralized enamel of 5304.30 square microns.....	72
FIGURE 32	Confocal, control, no area of demineralized enamel for all groups.....	73
APPENDIX I	SEM: Permanent tooth #1. Pit and fissure surface. Control. SEM: Permanent tooth #1. Pit fissure surface. Lased at 5 watts.....	98
APPENDIX II	SEM: Permanent tooth #1. Pit and fissure surface. Lased at 10 watts. SEM: Permanent tooth #1. Pit and fissure surface. Lased at 15 watts.....	99
APPENDIX III	SEM: Primary tooth #1. Pit and fissure surface. Lased at 20 watts. SEM: Primary tooth #1. Pit and fissure surface. Lased at 25 watts.....	100
APPENDIX IV	SEM: Permanent tooth #2. Pit and fissure surface. Pre laser treatment. SEM: Permanent tooth #2. Pit and fissure surface. Post laser treatment at 20 watts in same area as above picture. Area shows more roughness.....	101



APPENDIX V	SEM: Permanent tooth #3. Smooth surface. Control. Enamel prisms indicated. SEM: Permanent tooth #3. Smooth surface. Lased at 15 watts. Enamel appears smoother.....	102
APPENDIX VI	SEM: Permanent tooth #4. Pit and fissure. Control. SEM: Permanent tooth #4. Pit and fissure. Lased at 20 watts. Craters and cracking and smoother surface is noted.....	103
APPENDIX VII	SEM: Permanent tooth #5. Smooth surface. Control. SEM: Permanent tooth #5. Smooth surface. Lased at 20 watts. Surface is smoother.....	104
APPENDIX VIII	SEM: Permanent tooth #6. Pit and fissure. Control. Enamel prisms noted.....	105
APPENDIX IX	SEM: Primary tooth #2. Lased at 20 watts. Cracking and pitting noted.....	106
APPENDIX X	All surface characteristics seen on smooth surface in all four areas.....	107
APPENDIX XI	All surface characteristics seen on pit and fissure surfaces in all four areas.....	108

## INTRODUCTION



The laser is an important tool in modern medical practice. Its use in dentistry has been investigated for over three decades, and it has been approved by the Federal Drug Administration for soft tissue and temporomandibular joint surgeries. Recently, dental applications such as caries removal and fusion of enamel fissures has been advocated. The use of low energy density laser pulse such as the Neodymium:Yttrium, Aluminum, Garnet (Nd:YAG) and carbon dioxide lasers has been discovered to reduce the acid dissolution of enamel and partially inhibit artificial caries formation. Several explanations have been offered for this decrease in the solubility by Nelson, et al.<sup>1</sup> and Morioko and Oho.<sup>2</sup> One is that the lased surface is less permeable or porous for diffusion of ions into and out of enamel and dentin. Another suggestion is that compositional changes of enamel reduce its solubility to acid attack. Finally, it is possible that the organic matrix of the enamel is influenced by the laser radiation in such a way as to decrease its permeability.

Lasers may become the choice for removal of compromised tooth structure. All previous research has been done on permanent teeth with very little research on the effect of lasers on primary teeth. Chemical analysis on the enamel of primary teeth has shown an inorganic concentration between 92-93%, and the enamel of permanent teeth with a concentration of 95-96%.<sup>3</sup> Since lasers may affect the organic matrix and since primary teeth differ in their

composition from permanent teeth, further investigations are needed in this area.

The purpose of this study was to determine the effects of the Nd:YAG laser on the pits and fissures and smooth surfaces of human primary tooth enamel compared to the effects of the laser on similar enamel surfaces of the human permanent dentition. The hypothesis of the study is that the Nd:YAG laser induced changes on the enamel of primary teeth are similar to those induced on the enamel of permanent teeth with regard to acid solubility, resistance to caries-like lesion formation, surface topography and surface hardness.



## REVIEW OF LITERATURE

The laser is an important tool in modern medical practice, particularly as part of surgical intervention. Its use in dentistry has been investigated for over three decades. Lasers have Federal Drug Administration (FDA) approval for use in intraoral soft tissue, cutting and coagulating including the gingivae and oral mucosa. They do not have approval for use on hard tissue. Dental laser research in Pediatric Dentistry is expanding exponentially, and lasers may soon be used for the alteration of enamel structure to enhance resistance to caries.

Laser is an acronym for "light amplification by stimulated emission of radiation" (L.A.S.E.R.).<sup>4</sup> It is a beam of photons or energized atoms from within the electromagnetic spectrum which when focused in a thin beam can effectively cut almost anything.<sup>5</sup> The effect of laser exposure on target tissue is largely dependent on the wavelength, the absorption characteristic of the particular tissue, amount of power used, the length of exposure time, and whether the laser is in focus.<sup>4</sup> When the beam strikes tissue, it can be partially absorbed, transmitted, scattered or reflected. The absorbed laser energy vaporizes and carbonizes tissue most effectively.<sup>6</sup>

The reflected light bounces off the tissue surface and is directed outward. Therefore, the energy is dissipated very effectively, and there is little danger of damage to other parts of the mouth. Reflection limits the amount of energy that enters the tissue. The scattered energy occurs when it bounces from molecule to



molecule within the tissue and is affected by the degree of absorption, where high absorption minimizes scattering. Scattering distributes the energy over a larger volume of tissue, dissipating the thermal effects. Absorption is responsible for the thermal effects within the tissue, where light energy can be converted to heat energy. Transmission on the other hand is where the light energy can travel beyond a given tissue boundary and for this it must be quantified, and its effects should be considered. Absorption to a great extent controls the amount of reflection, scattering and transmission that occurs, and the wavelength is the primary determinant of absorption.<sup>7</sup>

Lasers are tissue specific and are categorized according to the type of medium used including Helium, Neon, Argon, Excimer and Carbon Dioxide. The carbon dioxide laser is most effective on soft tissue with high water content regardless of color and is highly absorbed by all biological tissues including enamel, dentin and fibrous soft oral tissue. Its lasing medium is carbon dioxide and is approved by the FDA for soft tissue use only. The Nd:YAG laser is most effective with pigmented tissues and is efficiently transmitted by water. It is approved by the FDA for soft tissue use also. The argon laser is more effective on pigmented tissue types or highly vascular tissues. It is also a good source for interproximal decay detection. The excimer laser has a halogen and a noble gas. It decomposes or breaks molecular bonds. Helium-neon lasers and the gallium-arsenide and gallium aluminum-arsenide are for soft tissue use only.



Some of the advantages for using lasers are: eliminating the use of local anesthesia, the decreased need for sutures, reduced surgical time and alleviation of the perceived threat from noisy rotating instruments. They lessen pain, and the level of fear is decreased. Treatment in multiple quadrants is possible without anesthesia. Post operative discomfort is decreased and there is minimal swelling or scarring. Lasers have no physical contact with the tissue, which leads to less mechanical trauma and little damage to adjacent tissues. They reduce the opportunity for blood borne contamination, which in turn creates the less likelihood of bacterial contamination of the operative site and the need for use of antibiotics in infection control will be decreased. Using lasers results in instantaneous sterilization of the wound site and hemostasis, yielding a dry field and a relatively bloodless surgery. This is beneficial in decreasing the risks of treating patients with coagulation or bleeding disorders and patients requiring prophylactic antibiotics. The laser is versatile in that it can cut, vaporize, ablate or coagulate. It can be used in the different specialties such as endodontics performing one visit root canals or periodontics for root planning and curettage.<sup>8</sup> It reduces the time utilized for certain procedures. In addition, lasers improve the practice image and its marketing due to high patient acceptance since patient management becomes less stressful.

Some of the disadvantages of the laser include the following. It is very costly, but with research and development it might become more cost effective. Generation of heat can be deleterious to the dental pulp and cause irreversible pulpal damage. Laser wounds do



not heal as rapidly compared to scalpel incisions, but they heal more rapidly than similar incisions made with an electrosurgical unit.

"Blind" procedures are contraindicated due to the potential damage to adjacent tissues, roots and osseous structures.

Lasers can cut, coagulate and vaporize tissues. Examples of some of its specific usages are the removal of fibrous gingival hyperplasia caused by Diphenylhydantoin (Dilantin Sodium). They have been successful and less traumatizing than the conventional gingivectomy procedures.<sup>4</sup> They have also been utilized in removing most white or red lesions appearing in the mouth, and numerous benign and premalignant lesions including fibromas, papillomas, pleomorphic adenomas, erosive lichen planus, inflammatory papillary hyperplasia, epulis, hemangiomas and pyogenic granulomas. They have also been used in crown lengthening procedures, removal of opercula, exposure of difficult eruption cases and exposure of implants. They have also been applied in frenectomy procedures, soft tissue grafts, excisional and incisional biopsies and gingivoplasties.<sup>9</sup>

No laser has been approved by the FDA for use in hard tissues, and few studies have been done on bone. Some of the areas previously studied include hard tissue desensitization, where 70-80% of laser sensitive roots have immediate and total cessation of discomfort. Another use investigated is the etching of pits and fissures using a staining medium such as India ink. Debonding of ceramic orthodontic brackets and pulpotomies on primary teeth have also been noted.<sup>10-12</sup> The laser creates a temporary analgesia effect on teeth, which would reduce or eliminate the use of local anesthesia.



This is believed to occur due to the energy interference with the sodium pump mechanism, changing the cell membrane permeability and/or temporarily altering the endings of the sensory axons.<sup>8</sup>

Vaporization of carious lesions in both enamel and dentin have been completed. Attempts at utilizing lasers for removal of carious lesions and preparation for restorative materials have met with moderate success. Initial investigations, such as Melcer, et al.<sup>13</sup> found that laser-treated carious dentin became sterile and resistant chemically and physically to demineralization. In addition, the exposure leads to an activation of the dentinogenesis process creating sclerotic dentin that can be detected by x-ray. They also showed that the pulp has no difficulty in withstanding the carbon dioxide laser beam. Myers and Myers<sup>14</sup> used the Neodymium:Yttrium-Aluminum-Garnet (Nd:YAG) laser for debridement of incipient caries. They removed pit and fissure enamel lesions in extracted teeth and later demonstrated the successful vaporization of small surface enamel lesions in vivo. They suggest, on the basis of their findings, that the Nd:YAG laser has potential to remove organic and inorganic debris from pits and fissures without causing pulpal or enamel injury. Nelson, et al.<sup>1</sup> also showed that the Carbon Dioxide laser was effective in removal of artificial caries. Since then, White, et al.<sup>15</sup> presented an in vivo study using a standard criteria to evaluate restorations and tooth vitality in patients who had received laser treatment three years previously. All teeth remained vital, asymptomatic, with no recurrent caries or periapical pathosis. The restorations placed after caries removal were intact and serviceable.



Another study<sup>16</sup> shows that the Nd:YAG laser applied to enamel for up to two minutes does not cause pulpal effects.

The alteration effects on tooth surface structure of lased teeth, where they become more resistant to dissolution, have received a great deal of attention. Numerous research studies have been done due to the potential damage to the oral soft tissues, the pulp, and to the enamel itself. The use of low energy density laser pulse has also been discovered to reduce the acid dissolution of enamel and partially inhibit artificial caries formation. In 1964, Goldman, et al.<sup>17</sup> made an attempt to replace the dental drill by the laser. He found that with sufficient energy densities the laser beam may penetrate sound enamel and the changes created are noticeable. He observed that the enamel on the sides of the lased areas showed no significant change; however, microscopic studies revealed selective deep destruction of colored carious areas from the laser impact.

Stern and Sognnaes<sup>18</sup> showed that the exposure of intact dental enamel to the laser beam caused a glasslike fusion of the enamel, which showed reduced birefringence under polarized light. They noted the limited pulpal temperature changes due to the high reflecting power of the dental enamel. Sognnaes and Stern in 1965<sup>19</sup> found that the enamel surface of extracted teeth exposed to the ruby laser had changed to demineralized subsurface with more resistance than that of preexposure. The superficial "glazing" exhibits a relatively greater resistance to demineralization. Stern, et al.<sup>20</sup> and Nelson, et al.<sup>21</sup> found inhibition of artificial lesion formation of up to fifty percent secondary to laser radiation of these surfaces. They noticed that the central melt region, although rough,



appeared to be covered by a thin, smooth, fused, glaze-like surface layer. Vahl<sup>22</sup> reported on structural changes in dental enamel due to laser irradiation. Micromorphological observations by the scanning electron microscope revealed a degree of destruction which depends on the condition of the structure of the dental hard tissue.

Yamamoto and Sato<sup>23,24</sup> found "remarkable" acid resistance in 73 percent of the specimens and "moderate" acid resistance in 27 percent of the specimens lased with Nd:YAG laser. The results suggested that the Nd:YAG laser irradiation could change the surface structure of enamel, making it more resistant to acid demineralization, an effective procedure for the prevention of dental caries. He also showed that inorganic products other than hydroxyapatite were not formed after irradiation with the Nd:YAG laser. Nelson, et al.<sup>25</sup> again studied the inhibitory effect on artificial lesion formation secondary to laser use. They suggested that low energy pulsed infrared laser radiation around the wavelengths of 9.3 micrometers represented near optimal conditions for lesion inhibition, confinement of the melt zone to a very thin surface zone and negligible damage to the pulp and oral mucosa. They also found that tetracalcium diphosphate monooxide was identified as being a component of the surface melt together with an apatite phase that had a reduced carbonate content when compared to normal surface enamel. Hicks, et al.<sup>26</sup> showed that the Argon laser irradiation resulted in significant reductions in body of the lesion depths following initiation and lesion progression periods. It also enhanced the ability of lased enamel to resist a constant in vitro cariogenic challenge. The explanation given was that the laser altered the pore



structure of lased enamel with entrapment and reprecipitation of mineral phases released during demineralization.

Vahl<sup>22</sup> and Kantola, et al.<sup>27</sup> analyzed laser effects by x-ray microanalysis and stated that the x-ray diffraction pattern of the enamel after the carbon dioxide laser or the ruby laser irradiation was altered with the production of tricalcium phosphate (TCP) instead of hydroxyapatite and the existence of alpha calcium orthophosphate. Kuroda and Fowler<sup>28</sup> showed that the laser irradiance melted the enamel apatite; the solidified melt was composed of minor phases of alpha-tricalcium phosphate, and tetracalcium phosphate and a major phase of modified apatite. The apatite modifications seen were 1) reductions in contents of water, protein and chloride, 2) no change in apatite hydroxide, 3) possible incorporation of oxide replacing some hydroxide ions, and 4) an uptake of traces of carbon dioxide and cyanate. Kantola<sup>29</sup> in another study showed zonal changes in the mineral concentration of the enamel. The changes were seen to take the form of a pressure-wave moving in the direction of the propagation of the laser beam. Changes in concentrations of calcium and phosphorous take place in the enamel of the lased tooth and follow a similar pattern, so that those areas in the preparation that show an increased calcium content also show an increased phosphorous content. Yamamoto and Ooya<sup>30</sup> showed that the greatest degree of demineralization occurred with the unlased normal enamel surface while the least demineralization was seen with the lased enamel irradiated at an energy density of  $20\text{j/cm}^2$ .



Fowler and Kurdo<sup>31</sup> said that along a steep temperature gradient, different compositional, structural and phase changes in tooth enamel are to be expected. A temperature gradient from 100 -1600 degrees was considered with subdivisions as follows: I, 100 - 650 degrees; II, 650 -1100 degrees, and III, > 1100 degrees. Two of the products formed in range III,  $\alpha$ - $\text{Ca}_3(\text{PO})_4$  and  $\text{Ca}_4(\text{PO}_4)_2\text{O}$ , and also identified in the fused-melted material from laser-irradiated tooth enamel are expected to markedly increase solubility in those regions that contain considerable amounts of these compounds. Products and changes occurring in range II, separate phases of  $\alpha$  and  $\beta$ - $\text{Ca}_3(\text{PO}_4)_2$  and a modified phase of apatite may increase or decrease the solubility depending on the Ca/P ratio and the resultant amounts of  $\alpha$  and  $\beta$ - $\text{Ca}_3(\text{PO}_4)_2$  formed. Modifications in tooth enamel apatite affected in range I are expected to decrease its solubility; the formation of pyrophosphate in this range may have a substantial effect on reducing the solubility rates.

Hashiguchi and Hashimoto<sup>32</sup> showed that at a lower level of energy there were no significant differences seen in the diffraction patterns between lased and unlased enamel. In scanning electron microscopic findings, there were no significant changes between lased and unlased enamel. When the unlased enamel and lased enamel were exposed to acid solution, unlased enamel showed a honeycomb pattern, while the lased enamel showed preferentially removed prism core material.

Two recent studies, Tagomori and Morioko<sup>33</sup> and Fox, et al.<sup>34</sup> have evaluated the combined effects of laser and fluoride treatment



on the acid resistance of enamel. It appears that the effect of fluoride and lasing of enamel are synergistic in their ability to increase lesion inhibition. Acidulated phosphate fluoride (APF) after irradiation caused a remarkable increase in acid resistance of the enamel, while APF application before laser irradiation showed a lesser effect. Sodium Fluoride (NaF) application caused less acid resistance and less fluoride uptake than APF application. It was found that the percent of the initial dissolution rate (IDR) of calcium, phosphorous and fluoride ions was greater for lased than for unlased enamel. It was also found by Borggreven, et al.<sup>35</sup> that the irradiation increased the permeability of enamel rather than decreased it, as was shown in other studies using sorbitol and glycerol.

Several explanations have been offered for the decrease in solubility by Nelson, et al.,<sup>21</sup> Ferreira, et al.,<sup>36</sup> and Oho and Morioka.<sup>2</sup> 1) The lased surface is sealed in the sense that it is less permeable or porous for diffusion of ions into and out of enamel or dentin during demineralization. 2) Laser induced compositional changes of enamel reduces its solubility to acid attack. 3) Formation of pyrophosphate or other chemical transformations during laser irradiation may inhibit dissolution. 4) The organic matrix of the enamel is influenced by the laser in such a way as to decrease its permeability. Findings discussed in the past showed that laser irradiation of intact human dental enamel inhibited artificial lesion formation. The contents of water, carbonate and organic substances were reduced in lased enamel. Gradual changes of birefringes were observed in lased enamel during treatment with acid solutions, and



these changes were attributed to mineralization of the microspaces and a rearrangement of ions in crystals in enamel that leads to a decreased lattice strain. With the higher energy treatment and increasing temperature, more effective results were obtained, and higher wavelengths showed 50 percent less demineralization than the controls. Fox, et al.<sup>34</sup> found that lased samples initially had a dissolution rate about 40 percent of that of the controls. After 10 minutes, the dissolution rate increased and became equal to that of the unlased enamel. This indicated that the mineral that had been effectively converted by the lasing process had dissolved, and the remaining unconverted mineral, therefore, behaved similarly to unlased enamel. The dissolution rate of lased enamel under conditions corresponding to a modest acid attack (pH 4.5-5.0) was expected to be much less than that of unlased enamel. This occurred due to a decrease in the driving force ion activity product. An alternative explanation for the dissolution rate reduction was that crystallite specific surface area or enamel permeability was reduced by laser irradiation.

Ferreira, et al.<sup>36</sup> using scanning electron microscopy showed extensive crazed enamel, and also crazed and cratered enamel following carbon dioxide laser irradiation. In conjunction with those findings, regions of rough exposed enamel were also seen due to the lifting off and removal of a top layer of crazed, or crazed and cratered, enamel. The type of induced change was mainly dependent on the energy density used, irradiation time, and enamel prism orientation. The crazed and cratered enamel had significant ultrastructural changes. New homogeneous and inhomogeneous



crystals of apatite with a different shape and larger size than those of the original and a loss of prismatic structure were observed. He also noted that there was no tetracalcium diphosphate or tricalcium phosphate detected. Stern and Sognnaes<sup>37</sup> also showed that CO<sub>2</sub> lasers created gross enamel surface alteration. Craze, porous and mottled, white surface opacities and light brown discolorations were seen. As laser energy decreased changes were less visible. There was no subsurface demineralization when a high energy laser was used. SEM observations showed that the exposed subsurface enamel appeared to have melted and resolidified. Hess<sup>38</sup> was able to grossly view a well-defined opaque surface that was clearly limited to all irradiated teeth. There was a sharp line of demarcation between the coated, irradiated area and the surrounding noncoated enamel surface. Using the SEM, the surface had melted and reformed with numerous small, bubble-like inclusions. Individual impact craters with shallow centers and raised edges containing numerous pores and large, bubble-like inclusions were noted.

As for microhardness, Ferreira, et al.<sup>36</sup> showed that both craze and craze and cratered enamel were consistently softer than adjacent unlased enamel, and that craze and cratered enamel was softer than craze enamel only. The surface alterations induced were dependent on the energy density used, on the angle at which the sample was initially sectioned with respect to the dental enamel junction and on the laser extent on the location of the lased area. Marquez, et al.<sup>39</sup> showed that the Knoop hardness had increased after laser exposure of teeth. Some explanations were given such as laser application can increase the mineralization by decreasing the



water and organic matter ratio with regard to the inorganic content and not affecting the mineral content. Hardness increases could also arise from the structural reorganization of the apatite crystals, or the laser produced small molecular changes in the mineral composition affecting the structure of each crystal or the interactions between themselves.

The above investigations have shown that laser irradiation of enamel at low energy densities increases its acid resistance. In addition, fusion of the lateral walls of enamel pits and fissures can occur with laser exposure, as well as removal of organic and inorganic debris from the pits and fissures. Since the early 1970s, the incidence of smooth surface caries in children has been reduced drastically. Through the use of various preventive measures, particularly fluoride in the water and other sources, the caries experience in pediatric populations has been greatly reduced<sup>40</sup>. Unfortunately, the impact of fluoride on the caries incidence on pit and fissure surfaces of teeth is minimal. Presently, 90 percent of the total caries experience in some pediatric populations now occurs within the pit and fissure grooves of the dentition.<sup>41</sup> Resin pit and fissure sealants have proven to be very effective at reducing the incidence of pit and fissure caries, however, resin sealants are very technique sensitive and require occasional replacement.

Several recent studies have sought to explain the use of the laser in decreasing acid dissolution of the pits and fissures of permanent enamel. Featherstone and Nelson<sup>42</sup> attributed the changes and alterations of enamel structure treated with lasers to enamel fusion. Variation in permeability, solubility, decalcification



resistance and mineral composition occur when the enamel absorbs enough energy from the laser beam. Quintana, et al.<sup>43</sup> explained the effects generated by the laser to the surface microfusion of enamel which depended on the total energy absorbed. The other possibility they presented was that the increase of the lased enamel resistance was structural and was originated by the amorphous reorganization of the enamel hydroxyapatite and the loss of the surface prismatic structure after its microfusion. Walsh and Perhan<sup>44</sup> utilized carbon dioxide lasers to fuse enamel pits and fissures. Their in vitro study examined the effect of focused infrared laser radiation on sound enamel and early pit and fissure caries. Low power levels (2 to 5 watts) induced localized melting and resolidification of enamel with little surface destruction. The lased enamel was opaque and exhibited a glaze-like lustre. For sound fissures, fusion of enamel from the lateral walls of the fissure eliminated the fissure space providing a sealing effect. While in carious fissures, the carious enamel was vaporized and adjacent sound enamel fused to partially eliminate the defects. They suggest that this technique has potential for sealing pits and fissures and producing physical, as well as chemical alterations in the enamel, which may have preventive benefits.

Synthetic hydroxyapatite has been successfully attached to enamel using infrared laser treatment to achieve sintering of the synthetic fissure sealant material by Stewart, et al.<sup>45</sup> Meurman, et al.<sup>46</sup> showed that synthetic hydroxyapatite, after laser treatment, was transformed to tricalcium phosphate, which is known to be more soluble than hydroxyapatite. If this same process occurs in dental



enamel, it is not beneficial. Studies need to be done with lower density energy lasers to achieve a beneficial result. He also observed no difference in the dissolution kinetics between unlased crude hydroxyapatite and Nd:YAG lased material, which indicated that neither the specific surface area nor the chemical composition were altered by irradiation with only Nd:YAG laser. The findings suggested that the HA-TCP transformation observed was mainly due to the CO<sub>2</sub> laser irradiation.

Arcoria, et al.<sup>47</sup> observed that different laser mediums produce enamel surface morphologies that are characteristic of a variety of inherent lasing parameters. In his experiment, surface enamel was irradiated with several lasers. Surface profile analyses were conducted with a profilometer to determine the amount of enamel surface roughness. The acid-etched samples exhibited both a greater amount of surface roughness and a qualitatively different type of enamel surface morphology than the laser-treated specimens. SEM observations indicated that laser ablations produced a wide variety of surface alterations and transformations ranging from a slightly roughened surface completely lacking in any cracks or fissures to a highly roughened, terraced or tiered surface possessing occasional cracks. The resultant enamel surface morphology was a function of the type of treatment performed.

A laser irradiated tooth enamel surface will have a temperature gradient that decreases towards the dentin junction. Dependent on irradiant conditions, the temperature may range from > 1400 degrees Celsius at the surface to near normal at the dentin-pulp junction.<sup>27</sup> Studies indicate, overall, that a temperature rise of



10 degrees Fahrenheit (approximately 6 degrees Celsius) can cause irreversible pulpal responses, and temperatures in excess of 20 degrees Fahrenheit (approximately 11 degrees Celsius) may cause necrosis of the pulp. Temperatures of this magnitude are known to occur during cavity preparation with uncooled burs and may occur during polishing and finishing of restorations.

von Fraunhofer and Allen<sup>48</sup> studied the heating effects at the dentinal pulpal wall on both buccal and lingual surfaces and showed an increase in heat as a function of the increase in power output from the laser. The temperatures measured at power levels 1-3W appeared to be of sufficient magnitude (exceeding 6 degrees) to cause at least localized pulpal inflammation and possible irreversible damage to the pulp tissue immediately opposite the site of laser irradiation. The study indicated that the observed temperature rise, for a given irradiation regimen, is determined by the tooth thickness with lower temperature rises associated with a greater dentin thickness. Other literature reported no pulpal damage with laser irradiation while others observed various degrees of damage, depending upon the laser used and the power setting of the laser.<sup>49-51</sup> Thermal effects of laser interaction with the tooth are dependent on the degree of energy absorption by the tooth. Laser absorption in turn is dependent on the wavelength of the specific lasing element, and in theory the thermal effects of laser energy absorption by the enamel would be low because enamel is an excellent light diffuser.

Dental laser research is expanding. Lasers may become an armamentarium for the alteration of enamel structure to enhance resistance to caries. The potential of laser radiation to achieve fusion

of pit and fissure enamel and the elimination of any morphological defects still needs exploration. Lasers may become the method of choice for removal of compromised tooth structure and in time be involved in the restorative process by fusing a semisynthetic hydroxyapatite material to the normal tooth structure. Finally, most of the studies that have been done on human enamel to date have been with the permanent dentition. As the use of lasers on hard tissues increases, it would be useful to study the decrease in acid dissolution in lasered enamel in the primary dentition, especially since laser effects depend on the enamel prisms, organic and inorganic structure and energy, and since primary enamel does differ from permanent enamel.



## METHODS AND MATERIALS

Disassociated teeth were collected from pediatric dentists in the Indianapolis area and the department of oral surgery at Indiana University School of Dentistry. The teeth were initially stored in formalin, and after they had been cut, they were stored in tap water up to and during the course of the study. The teeth collected were primary molars, exfoliated or extracted due to ankylosis or for orthodontic reasons, and permanent molars that had also been extracted. All teeth were noncarious and nonrestored.

The laser equipment used was the Endo Technic laser-35 (see Figure 1), which was provided by Laser Medical Technologies, currently known as BioLaze. The laser is a Neodymium:Yttrium, Aluminum, Garnet laser (Nd:YAG) with a wavelength of 1064 nm, intended for all intraoral soft tissue applications. It is a 50 Hz pulsed, solid state laser that generates a 0.8 millisecond pulse with adjustable power from 3 to 25 watts (105-500 millijoule) and has a beam divergence of 5 mrad. The laser output is managed by a fiber optic system through contact and non-contact handpieces that accomodate different diameter fibers. The fiber diameter for the contact handpiece is a cylindrical tip configuration that ranges between 300-600 microns. The energy fluence ranges between 7.0 J/mm<sup>2</sup> (max) to 0.4 J/mm<sup>2</sup> (min) at the focal point that is 1.5 cm from the end of the handpiece tip. A visible non-laser aiming beam is employed for targeting. The fiberoptic housing contains an air and water line. Water and air spray were utilized in this study with



water pressure of 2.0 psi and air pressure of 20 psi to control the zone of effect on the target tissue.

A pilot study was performed to determine if a contact or a non-contact hand piece should be used and at what strength. The laser was used with various watts from 5-25 going up in five watt increment intervals. Each tooth was divided into eight areas, four areas to lase at the different powers and four areas non-lased in approximation to the others to try and eliminate the variation from one tooth to another. However, there were still variations. There were too many variations and no consistent findings for any specific power recommendations. (See Appendixes I-V. All magnifications were at X2000.) The alterations on the surface included charring, which was visible clinically with the naked eye, and under the SEM the changes included pitting, cratering, cracking and alteration in prism structure. It was decided to use the three watts recommended by the company to best meet this study's objectives.

In this study, four parameters involving the effect of the Nd:YAG treatment of primary and permanent enamel were evaluated:

- 1) Scanning electron microscopic comparison of lased and non-lased enamel.
- 2) Enamel acid resistance as measured by calcium and phosphorous release.
- 3) Vicker's Hardness of the lased and non-lased enamel.
- 4) Confocal microscopic examination of caries-like lesion formation on lased and non-lased enamel.

### Parameter #1-SEM evaluation

In this part, eight primary and eight permanent specimens were taken, four from the smooth surface and four from the occlusal surface for each tooth type. Each specimen was cut down to a small round specimen then mounted on a plastic cylindrical lucite rod (see Figure 2). Half of each specimen was lased with 3.0 watts per the recommendation of the laser company. The contact handpiece with 600 micron diameter was used with gentle pressure applied with gentle stroking action (see Figure 3). The lasing method involved strokes up and down the specimen until the entire surface was treated. This was determined by viewing the beam visually where the strokes resembled the motion of a handpiece in the mouth. The lasing technique took between 5-10 seconds depending on the specimen size (depending if half or all the chip was lased). The surfaces were lased only once according to the observation with the naked eye.

After lasing, the chip was covered perpendicular to the lased area with red nail varnish and was exposed to an acid challenge using 0.1 M lactic acid, 50 percent saturated HAP and 0.2 percent Carbopol, at pH 5.0 for 48 hrs at 37 degrees Celsius. This provided four areas: 1) sound covered area, 2) sound, acid demineralized area, 3) lased covered area, and 4) lased acid demineralized area (see Figure 4). After etching, the chips were rinsed with deionized water and stored to dry. The specimens were removed from the rods, the nail varnish cleaned with acetone and all chips were mounted on spurs to be plated with a gold/palladium layer.



A layer of Au/Pd: 60/40, 10-20 nm in thickness was sputtered on the chips and examined with the Hitachi S450 microscope at 20 KV with X2000 magnification. Some of the smooth specimens were looked at with X10k magnification to see if there was any change. This enabled the assessment of surface changes that were suggestive of enamel fusion and the degree of roughness created. It also showed if there were any differences between the craters and crazes formed in the primary and permanent enamel.

#### Parameter #2-Enamel Acid Resistance

The second parameter determined if the laser treatment had an effect on acid resistance as measured by calcium and phosphate release. Twenty primary and twenty permanent, 3 mm round chips were taken from the smooth surfaces. Using a 3 mm in diameter cutting cylinder (see Figures 5-6), round chips were cut and mounted on a lucite rod with pink denture acrylic (Figure 2). After mounting, each chip was ground to have a smooth and flat surface area with a 600u grit of carbimet paper disc. Each chip was highly polished using a GE Silicone pad with a Gamma micropolish Alumina B #3 paste with a particle size of 0.05 microns to give the enamel a luster and a natural look (see Figure 7). Each one was inspected to confirm that the surface area was all enamel.

Ten of each of the permanent and primary teeth were lased with the same method as stated above. All 40 chips were then taken and acid etched using 0.5 ml of 1.0 M of prechloric acid ( $\text{HClO}_4$ ), for 15 seconds according to Indiana University Oral Health Research Institute (OHRI) laboratory test procedures. The dissolution of



enamel in acid yielded free calcium and phosphorous ( $\text{PO}_4$ ) ions. After all the specimens had been decalcified, the samples were rinsed with deionized water. The solution was then taken to be analyzed for the free ions. Determining the amount of calcium and phosphorous released by enamel to an acid buffer as a function of time, under controlled conditions, provides a good indication of the enamel solubility rate.

Calcium ions were analyzed by using the standard atomic absorption method following OHRI guidelines. Preparation of a standard curve was done before the assay of test samples were run. One ml of stock calcium standard was placed into a 100 ml volumetric flask and diluted with deionized water to yield 25 ppm Ca solution. Standards containing 0.0 and 5.0 ppm were prepared in 50 ml volumetric flasks using 0.0 ml and 10 ml of 25 ppm Ca solution, respectively. To each flask, 10 ml of 5 percent Lanthum in 25 percent HCl were added. Deionized water was added to the solution to make it 25 ml.

For preparing the decalcified samples, 50 ul of 1 N  $\text{HClO}_4$  used in etching were placed in a 10 ml flask. One ml of lanthum chloride and 3.95 ml deiodized water were added. When all these were prepared, an atomic absorption spectrophometer equipped with a digital concentration readout was used. The standard curve was calibrated, and then each sample was assayed and concentrations recorded. The machine was restandarized after each 5 samples used.

The phosphorous ions were analyzed using the method of Fiske and Subbarow<sup>52</sup> which involves quantitating the color change



of the reduction of phosphomolybdic acid by 1,2,6-aminonaphtholsulfonic acid. A standard curve was prepared by using 0.25 ml of 1N perchloric acid ( $\text{HClO}_4$ ), 2.5 ml of Ammonium Molybdate ACS assay, standard phosphate solution at different concentrations and 1 ml of 1 Amonia, 2 Naphthal, 4 Sulfonic Acid (ANSA) and was brought up to 25 ml with deionized water. These were placed in the colorimeter and a curve established. The samples were prepared by taking 0.25 ml of the perchloric used to etch, 2.5 ml of molybdate and 1 ml of ANSA, and the solution was equilibrated to 25 ml with deionized water. Each solution was poured in the colorimetric tube twice: the first time to rinse the previous solution out and the second time to read the phosphorous.

### Parameter #3-Vicker's Hardness Test

The third parameter studied was Vicker's Hardness. Twenty primary and twenty permanent chips were used and prepared as in parameter #2. The surface hardness of each specimen was determined using Vicker indents with a 200 g load (see Figures 8-10) and measured on the computer screen. Since there are many variations of hardness on every specimen, four hardness tests were done at four different locations; at the 2, 4, 8 and 10 o'clock positions, and the average was taken (see Figure 11). After all 40 specimens were tested prior to any treatment (reading 1), laser treatment was done on 10 primary chips and 10 permanent chips. All 40 specimens had a second hardness test (reading 2) in an approximate location to the first indents (see Figure 11). Finally, all the chips were soaked with 10 ml of demineralizing solution using 0.1 M lactic acid, 50



percent saturated HAP and 0.2 percent Carbopol, at pH 5.0 for 48 hours. The Carbopol solution was used instead of just acid to simulate plaque acid attack in which a concentration gradient of calcium and phosphorous is at the enamel surface. A final hardness test (reading 3) was then completed on all 40 chips.

#### Parameter #4-Caries-like Lesion Formation

In this section, the specimens from parameter #3 were prepared for Confocal Microscopic observation using the Noran Odyssey microscope. All chips were cut in half with a fine diamond disc, perpendicular to the surface (see Figure 12). Rhodamine B at 0.1 mM solution was then placed on all the cross-sectioned surfaces for approximately 12 hours. The cross sections were then observed at 488 nm excitation with a 515 nm barrier filter. Thresholding using pixel values starting with 255 and then decreasing it until the created lesion was the only thing seen. The artificially induced caries lesion depth was then calculated on all 40 chips by taking the average of two readings on each chip. One field from the edge on each side, the left and right, equal to 500 microns in length was measured and then a reading was taken from that point towards the center of the chip. This was done for both sides. A 250 micron area was then taken as a representative of the lesion depth in that image. The Images were captured using image-I software (Universal Imaging Corp, Westchester, Penn.) and printed using a color videoprinter and slides.



## RESULTS

### Pilot Study

The results from this pilot study were not consistent, and a useful power level recommendation for the laser could not be determined. There were many variations in the surface for both the lased and non-lased teeth, and so results are not included in this section (see Appendixes I-IX). (All magnifications were at X2000.)

### Parameter #1-SEM evaluation

The observations seen with the SEM were qualitatively analyzed. Many observations were noted: pitting, cracking, enamel prisms, white bubbles, smooth surface, rough surface, grainy appearance, cobble stone appearance, ridges, ravines, cratering and particles. Different chips showed varied degrees of surface alteration. All these qualities were seen on both the primary and permanent chips with no pattern for any specific area. A pattern could not be found for any particular combination of treatment done on the chips. All magnifications were done at X1000.

All four areas on the smooth surface chips of the lased and non-lased teeth appeared to have varied degrees of smooth surfaces with the exception of one primary chip that showed severe roughness and scratches (see Figures 13-17 and Appendix X). The pit and fissure chips showed a wider variety of surface characteristics. For these chips, there was also no specific pattern that could be identified with a location. No difference was seen for



the type of tooth either. The permanent and primary teeth responded similarly. The qualities noted on these were: pitting, cratering, cracking, enamel prisms, white bubbles, smooth surface, rough surface, grainy appearance, cobble stone surface, ridges and ravines, all seen at X1000 magnification (see Figures 18-27 and Appendix XI).

#### Parameter #2- Enamel Acid Resistance

The results for calcium ion disassociation following acid attack for permanent lased teeth had a mean of  $1.81 \text{ ppm} \pm 0.51$ , and for non-lased permanent the mean was  $1.85 \text{ ppm} \pm 0.36$ . For primary lased teeth the mean was  $2.34 \text{ ppm} \pm 0.43$ , and for non-lased the mean was  $2.90 \text{ ppm} \pm 0.36$  (see Table I).

The phosphorous disassociation following acid attack for the permanent lased teeth had a mean of  $49.90 \text{ ppm} \pm 11.71$ , and for non-lased permanent teeth the mean was  $56.40 \text{ ppm} \pm 14.12$ . As for the primary lased teeth, the mean was  $80.50 \text{ ppm} \pm 15.99$ , and for non-lased teeth the mean was  $89.80 \text{ ppm} \pm 9.26$ , see Table I. (Tables IA and IB have individual readings of each specimen.)

The effects of type of tooth (permanent or primary) and whether the tooth was lased or non-lased (L/NL) on calcium and phosphorous release were analyzed using analysis of variance. Multiple comparisons were conducted using Fisher's Protected Least Significant Differences at an overall confidence level of 95 percent. The analysis for calcium release showed that the interaction between type of tooth and L/NL was significant ( $p=0.0126$ ). The permanent teeth calcium dissolution was the same for the lased and the non-



lased, but for the primary teeth the calcium dissolution was higher for the non-lased. This means that there was a difference in calcium dissociation depending if the tooth was lased or non-lased and if the tooth was permanent or primary.

At the same time the calcium release was higher for the primary teeth compared to the permanent regardless if they were lased or not; therefore, the type of tooth had a significant effect on calcium release ( $p=0.0001$ ). Eliminating the variable of the type of tooth knowing that the primary teeth have a greater dissolution rate, the laser affected the primary teeth differently. It made the dissolution rate decrease in all the lased primary teeth but not the lased permanent. The interaction of the type of tooth and L/NL was significant ( $p=0.0126$ ).

The analysis of phosphorous release showed that the type of tooth had a significant effect ( $p=0.0001$ ). It was significantly higher for primary teeth than for permanent teeth. L/NL had a significant effect on phosphorous release also ( $p=0.0111$ ). It was significantly higher for non-lased than for lased. The interaction between type of tooth and L/NL was not significant for phosphorous release since both permanent and primary teeth acted similarly after laser exposure, and the change was the same for both.

### Parameter #3- Vicker's Hardness Test

The surface hardness analysis studied the effects of type of tooth (permanent or primary) and whether the tooth was lased or non-lased (L/NL) on tooth hardness (measured by VHN). It was analyzed using analysis of variance. Multiple comparisons were



conducted using Fisher's Protected Least Significant Differences at an overall confidence level of 95 percent.

Analysis of reading 1 (Pretreatment): Since all teeth were non-lased at reading 1, only type of tooth was compared. The mean for permanent NL was 346.15 VHN  $\pm$  10.95, and for primary NL teeth the mean was 321.12 VHN  $\pm$  19.97 (see Tables II, IIA and IIB for individual readings). The type of tooth had a significant effect on tooth hardness ( $p=0.0001$ ). The hardness was significantly higher for permanent teeth than for primary teeth.

Analysis of reading 2 (half of the primary and permanent teeth non-lased and half-lased): The mean readings for permanent lased was 364.01 VHN  $\pm$  10.25, and for non-lased the mean was 351.08 VHN  $\pm$  7.38. As for primary lased teeth, the mean was 326.32 VHN  $\pm$  11.0024, and for non-lased the mean was 311.18 VHN  $\pm$  17.25 (see Tables II, IIC and IID for individual readings).

The type of tooth had a significant effect on tooth hardness ( $p=0.0001$ ). The hardness was significantly higher for permanent teeth than for primary teeth. L/NL had a significant effect on tooth hardness ( $p=0.0007$ ). Hardness was significantly higher for lased permanent teeth than for non-lased permanent teeth but just the opposite for primary teeth. The lased primary teeth were not as hard as the non-lased primary teeth; they became softer.

Analysis of reading 3 (all 40 specimens treated): The VHN measurements for reading 3 did not follow a normal distribution so analyses were performed on  $\log(\text{VHN})$ , (see Tables II, IIE and IIF for individual readings). Neither type of tooth ( $p=0.4960$ ) nor L/NL ( $p=0.4476$ ) had a significant effect on tooth hardness.



Comparative analysis: Another way of analyzing this data was to perform the analyses on the differences. Reading 1 is a "baseline" measurement, so by subtracting the VHN measurement for reading 1 from the VHN measurements for readings 2 and 3, we can adjust for initial differences in the chips. An analysis was also done on the difference between readings 2 and 3 (reading 3 minus reading 2) to see if there were any changes in the way type of tooth or L/NL interaction affected tooth hardness between readings 2 and 3.

Analysis of reading 2 (difference from baseline): the interaction between type of tooth and L/NL was significant ( $p=0.0001$ ), see Tables III and IIIA. For lased teeth, the permanent teeth had a significantly larger difference from baseline than primary teeth. The hardness for permanent teeth increased from baseline to reading 2, and the hardness for primary teeth decreased from baseline to reading 2. For permanent teeth, lased teeth had a significantly larger difference from baseline than non-lased teeth. The VHN for lased teeth increased from baseline to reading 2, and VHN for non-lased teeth decreased from baseline to reading 2. For primary teeth, non-lased teeth had a significantly larger difference from baseline than lased teeth. Hardness for lased teeth decreased from baseline to reading 2, and hardness for non-lased teeth increased from baseline to reading 2.

Analysis of reading 3 (difference from baseline): The distribution of difference #2 = (VHN for reading 3 minus VHN for reading 1) was not normal. The analysis was instead performed on  $\log(\text{diff2} + 400)$ , see Tables III and IIIA.



The interaction between type of tooth and L/NL was significant ( $p=0.0023$ ). For lased teeth, difference from baseline was not significantly different for permanent and primary teeth. For non-lased teeth, permanent teeth had a significantly larger difference from baseline than primary teeth. VHN for permanent teeth decreased from baseline to reading 3. VHN for primary teeth also decreased from baseline to reading 3, but it did not decrease as much as for permanent teeth.

For permanent teeth, non-lased teeth had a significantly larger difference from baseline than lased teeth. The hardness for non-lased teeth decreased from baseline to reading 3, and the hardness for lased teeth had also decreased from baseline to reading 3, but it did not decrease as much as for non-lased teeth. For the primary teeth, lased teeth had a significantly larger difference from baseline than non-lased teeth. The hardness for lased and non-lased primary teeth decreased from baseline to reading 3. However, the non-lased did not decrease as much as the lased teeth.

Analysis of difference between reading 2 and reading 3: The distribution of difference #3 = (VHN for reading 3 minus VHN for reading 2) was not normal. The analysis was instead performed on  $\log(\text{diff3} + 400)$ , see Tables III and IIIA.

Laser treatment of the teeth did not have a significant effect on hardness ( $p=0.4201$ ). L/NL does not cause any changes in hardness between readings 2 and 3. However, the type of tooth had a significant effect on hardness ( $p=0.0003$ ). Permanent teeth had a significantly larger difference in hardness than primary teeth. The hardness for permanent teeth decreased from reading 2 to reading 3,

and the hardness for primary teeth also decreased from reading 2 to reading 3 but not as much as for permanent teeth.

Parameter #4 - Caries like lesion formation:

As for the Confocal analysis of lesion depth, two 250 micron areas were taken on each chip, one from the left and one from the right side, and an average was calculated (see Figures 28-31). The effects of type of tooth (permanent and primary) and whether the tooth was lased or non-lased (L/NL) on averaged confocal microscope readings were analyzed using analysis of variance. Multiple comparisons were conducted using Fisher's Protected Least Significant Differences at an overall confidence level of 95 percent.

The lased and non-lased variable did not have a significant effect on the confocal microscope readings ( $p=0.4108$ ). As for the type of tooth, there was a significant difference ( $p=0.0124$ ). The primary teeth had significantly higher readings than permanent teeth indicating deeper lesions, see Tables IV and IVA.



## TABLES AND FIGURES

TABLE I

Statistical analysis of calcium and phosphorous dissassociation for  
lased (L)/non-lased (NL) permanent (Per) and primary (Pri) enamel.

Type	L/NL	# Obs	Variable	#	Mean ppm	Std Dev	Std Error	Minimum	Maximum
Per	L	10	Calcium	10	1.81	0.51	0.16	1.08	2.44
Per	NL	10	Calcium	10	1.85	0.36	0.11	1.30	2.48
Pri	L	10	Calcium	10	2.34	0.43	0.14	1.91	3.21
Pri	NL	10	Calcium	10	2.90	0.36	0.11	2.32	3.43
Per	L	10	Phosphorus	10	49.90	11.71	3.70	36.00	76.00
Per	NL	10	Phosphorus	10	56.40	14.12	4.47	33.00	82.00
Pri	L	10	Phosphorus	10	80.50	15.99	5.06	61.00	111.00
Pri	NL	10	Phosphorus	10	89.80	9.26	2.93	79.00	104.00



TABLE IA				
The individual readings for phosphorous and calcium dissassociation for lased (L)/non-lased (NL) permanent (Per) teeth.				
Per/Pri	#	L/NL	Calc	Phos
Per	1	L	1.53	46.00
Per	1	NL	1.57	51.00
Per	2	L	1.42	41.00
Per	2	NL	1.74	51.00
Per	3	L	2.22	50.00
Per	3	NL	1.64	56.00
Per	4	L	1.15	39.00
Per	4	NL	1.54	52.00
Per	5	L	1.08	36.00
Per	5	NL	1.30	33.00
Per	6	L	1.67	54.00
Per	6	NL	2.24	45.00
Per	7	L	1.98	48.00
Per	7	NL	1.91	54.00
Per	8	L	2.35	48.00
Per	8	NL	2.00	68.00
Per	9	L	2.44	61.00
Per	9	NL	2.48	82.00
Per	10	L	2.30	76.00
Per	10	NL	2.10	72.00

TABLE IB				
The individual readings for phosphorous and calcium dissassociation for lased (L)/ non-lased (NL) primary (Pri) teeth.				
Per/Pri	#	L/NL	Calc	Phos
Pri	1	L	2.02	67.00
Pri	1	NL	2.32	81.00
Pri	2	L	2.84	97.00
Pri	2	NL	2.61	79.00
Pri	3	L	2.30	80.00
Pri	3	NL	3.24	100.00
Pri	4	L	2.10	70.00
Pri	4	NL	2.57	80.00
Pri	5	L	1.91	61.00
Pri	5	NL	2.95	92.00
Pri	6	L	2.33	82.00
Pri	6	NL	3.04	94.00
Pri	7	L	1.97	67.00
Pri	7	NL	2.77	82.00
Pri	8	L	2.07	75.00
Pri	8	NL	3.43	99.00
Pri	9	L	3.21	111.00
Pri	9	NL	2.80	87.00
Pri	10	L	2.68	95.00
Pri	10	NL	3.30	104.00



TABLE II

Statistical analysis of the Vicker's Hardness Test of all chips  
[permanent (Per), primary (Pri), lased (L), non-lased (NL)].

Reading	Type	L/NL	# Obs	Variable	#	Mean ppm	Std Dev	Std Error	Minimum	Maximum
1	Per	NL	20	VHN	20	346.15	10.95	2.45	322.00	362.35
				SD	20	13.93	7.05	1.58	3.38	29.34
	Pri	NL	20	VHN	20	321.12	19.97	4.47	284.70	361.10
				SD	20	18.41	12.93	2.89	5.30	55.99
2	Per	L	10	VHN	10	364.08	10.25	3.24	345.70	377.80
				SD	10	14.88	6.43	2.03	1.72	25.02
		NL	10	VHN	10	351.08	7.38	2.34	342.20	362.55
				SD	10	10.92	6.22	1.97	3.91	24.53
	Pri	L	10	VHN	10	326.32	11.00	3.48	303.65	336.70
				SD	10	12.89	7.48	2.37	7.52	29.92
		NL	10	VHN	10	311.18	17.25	5.45	287.10	338.10
				SD	10	23.73	19.29	6.10	8.00	65.95
3	Per	L	10	VHN	10	55.70	22.66	7.17	32.30	100.95
				SD	10	27.03	19.50	6.17	4.77	64.36
		NL	10	VHN	10	39.64	19.75	6.25	19.13	72.48
				SD	10	14.02	14.15	4.47	2.83	43.56
	Pri	L	10	VHN	10	43.99	37.28	11.79	17.03	143.85
				SD	10	26.39	30.70	9.71	1.53	90.35
		NL	10	VHN	10	48.68	34.24	10.83	24.28	128.18
			10	SD	10	38.93	28.54	9.03	4.72	79.15



**TABLE IIA**  
**All four individual readings of Vicker's Hardness Test**  
**reading #1 — control**  
**lased (L) and non-lased (NL) permanent (Per) chips.**

	Hardness	L/NL	Chip #	VHN1	VHN2	VHN3	VHN4
Per	1	NL	1	344.80	334.40	357.60	320.80
Per	1	NL	2	317.00	360.00	351.00	362.20
Per	1	NL	3	357.60	332.40	338.60	346.00
Per	1	NL	4	332.40	338.60	342.60	326.60
Per	1	NL	5	364.40	346.80	299.40	357.60
Per	1	NL	6	315.20	315.20	319.00	338.60
Per	1	NL	7	338.60	338.60	364.40	346.80
Per	1	NL	8	336.40	334.40	324.60	324.60
Per	1	NL	9	304.40	355.40	324.60	342.60
Per	1	NL	10	364.40	360.00	362.20	353.20
Per	1	NL	11	360.00	371.40	355.40	349.00
Per	1	NL	12	381.00	353.20	338.60	357.60
Per	1	NL	13	369.00	366.80	346.80	366.80
Per	1	NL	14	369.00	334.40	326.60	330.40
Per	1	NL	15	334.40	381.00	330.40	349.00
Per	1	NL	16	353.20	360.00	357.60	353.20
Per	1	NL	17	344.80	351.20	362.20	346.80
Per	1	NL	18	349.00	381.00	355.40	340.60
Per	1	NL	19	351.20	322.80	334.40	369.00
Per	1	NL	20	340.60	362.20	346.80	344.80



<p><b>TABLE IIB</b>  <b>All four individual readings of Vicker's Hardness Test</b>  <b>reading #1 — control</b>  <b>lased (L) and non-lased (NL) primary (Pri) chips.</b></p>							
	Hardness	L/NL	Chip #	VHN1	VHN2	VHN3	VHN 4
Pri	1	NL	1	328.60	324.60	344.80	364.40
Pri	1	NL	2	340.60	311.60	313.40	336.40
Pri	1	NL	3	353.20	420.40	334.40	336.40
Pri	1	NL	4	324.60	320.80	289.40	332.40
Pri	1	NL	5	344.80	311.60	317.00	326.60
Pri	1	NL	6	381.00	332.40	338.60	344.80
Pri	1	NL	7	308.00	342.60	324.60	332.40
Pri	1	NL	8	319.00	332.40	336.40	360.00
Pri	1	NL	9	317.00	313.40	304.40	315.20
Pri	1	NL	10	342.60	342.60	342.60	353.20
Pri	1	NL	11	292.60	287.80	275.40	283.00
Pri	1	NL	12	336.40	249.00	291.00	330.40
Pri	1	NL	13	332.40	338.60	302.80	346.80
Pri	1	NL	14	306.20	332.40	320.80	328.60
Pri	1	NL	15	326.60	324.60	311.60	326.60
Pri	1	NL	16	226.20	351.20	326.60	330.40
Pri	1	NL	17	283.00	272.40	291.00	308.00
Pri	1	NL	18	294.20	326.60	299.40	317.00
Pri	1	NL	19	308.00	304.40	292.60	275.40
Pri	1	NL	20	334.40	315.20	311.60	319.00



<p>TABLE IIC</p> <p>All four readings of Vicker's Hardness Test</p> <p>reading #2</p> <p>after lasing of permanent (Per) chips.</p>							
	Hardness	L/NL	Chip #	VHN1	VHN2	VHN3	VHN4
Per	2	L	1	364.40	355.40	381.00	371.40
Per	2	L	2	338.60	381.00	396.00	360.00
Per	2	L	3	388.40	353.20	391.00	378.60
Per	2	L	4	3860.00	366.80	378.60	366.80
Per	2	L	5	357.60	344.80	322.80	357.60
Per	2	L	6	342.60	366.80	373.80	349.00
Per	2	L	7	344.80	381.00	364.40	369.00
Per	2	L	8	353.20	351.20	355.40	353.20
Per	2	L	9	330.40	360.00	357.60	381.00
Per	2	L	10	366.80	398.60	360.00	364.40
Per	2	NL	11	357.60	355.40	360.00	349.00
Per	2	NL	12	353.20	346.80	357.60	366.80
Per	2	NL	13	353.20	362.20	338.60	328.60
Per	2	NL	14	355.40	353.20	346.80	349.00
Per	2	NL	15	357.60	364.40	353.20	366.80
Per	2	NL	16	360.00	342.60	369.00	378.60
Per	2	NL	17	334.40	346.80	364.40	355.40
Per	2	NL	18	334.40	349.00	355.40	336.40
Per	2	NL	19	371.40	319.00	326.60	355.40
Per	2	NL	20	330.40	349.00	342.60	346.80



<p style="text-align: center;">TABLE IID</p> <p style="text-align: center;">All four readings of Vicker's Hardness Test</p> <p style="text-align: center;">reading #2</p> <p style="text-align: center;">after lasing of primary (Pri) chips.</p>							
	Hardness	L/NL	Chip #	VHN1	VHN2	VHN3	VHN4
Pri	2	L	1	338.60	340.60	322.80	344.80
Pri	2	L	2	308.00	315.20	313.40	330.40
Pri	2	L	3	328.60	344.80	330.40	338.60
Pri	2	L	4	328.60	344.80	330.40	338.60
Pri	2	L	5	340.60	319.00	301.00	328.60
Pri	2	L	6	346.80	326.60	336.40	334.40
Pri	2	L	7	313.40	334.40	328.60	330.40
Pri	2	L	8	330.40	344.80	324.60	326.60
Pri	2	L	9	315.20	319.00	308.00	272.40
Pri	2	L	10	334.40	276.80	344.80	317.00
Pri	2	NL	11	291.00	308.00	284.60	287.80
Pri	2	NL	12	330.40	265.20	304.40	355.40
Pri	2	NL	13	334.40	330.40	338.60	349.00
Pri	2	NL	14	336.40	200.60	336.40	322.80
Pri	2	NL	15	334.40	349.00	309.80	342.60
Pri	2	NL	16	247.60	336.40	340.60	319.00
Pri	2	NL	17	280.00	268.00	289.40	311.00
Pri	2	NL	18	324.60	332.40	313.40	311.60
Pri	2	NL	19	297.60	302.80	301.00	284.60
Pri	2	NL	20	336.40	330.40	297.60	311.60



**TABLE IIE**  
**All four readings of Vicker's Hardness Test**  
**reading #3**  
**after acid etching of permanent (Per) chips.**

	Hardness	L/NL	Chip #	VHN1	VHN2	VHN3	VHN4
Per	3	L	1	47.10	36.90	41.80	46.60
Per	3	L	2	27.10	49.20	41.40	23.20
Per	3	L	3	87.50	30.70	35.40	55.50
Per	3	L	4	60.90	104.40	42.50	33.60
Per	3	L	5	91.10	75.00	143.70	94.00
Per	3	L	6	20.60	36.50	46.90	25.20
Per	3	L	7	23.70	45.30	34.50	90.20
Per	3	L	8	30.10	30.90	26.60	44.50
Per	3	L	9	22.10	147.70	92.50	60.80
Per	3	L	10	165.70	31.20	31.10	54.20
Per	3	NL	11	28.90	21.10	27.30	23.00
Per	3	NL	12	109.50	77.00	65.20	38.20
Per	3	NL	13	32.00	29.40	20.10	29.70
Per	3	NL	14	36.90	38.20	34.70	26.10
Per	3	NL	15	61.90	35.20	38.80	82.10
Per	3	NL	16	28.90	31.00	23.00	25.90
Per	3	NL	17	<16.4	18.50	18.50	23.10
Per	3	NL	18	26.00	21.10	<15.7	19.20
Per	3	NL	19	42.20	123.50	81.80	26.10
Per	3	NL	20	33.50	39.10	77.40	39.40



**TABLE IIF**  
**All four readings of Vicker's Hardness Test**  
**reading #3**  
**after acid etching of primary (Pri) chips.**

	Hardness	L/NL	Chip #	VHN1	VHN2	VHN3	VHN4
Pri	3	L	1	45.50	<17.0	30.10	<17.3
Pri	3	L	2	<17.9	36.90	59.50	<19.8
Pri	3	L	3	<15.7	22.00	23.70	43.50
Pri	3	L	4	<18.2	<15.7	<15.7	18.50
Pri	3	L	5	138.80	41.10	179.10	216.40
Pri	3	L	6	20.60	18.80	54.50	32.10
Pri	3	L	7	<17.1	34.60	23.40	18.90
Pri	3	L	8	<16.1	19.10	21.70	199.60
Pri	3	L	9	33.70	31.40	18.20	71.90
Pri	3	L	10	30.80	39.70	30.40	34.50
Pri	3	NL	11	103.00	146.00	45.60	32.90
Pri	3	NL	12	76.30	233.00	144.80	58.60
Pri	3	NL	13	<18.7	32.90	25.50	24.10
Pri	3	NL	14	<17.6	38.40	23.70	24.20
Pri	3	NL	15	23.40	53.30	86.70	19.80
Pri	3	NL	16	171.50	47.70	21.20	24.20
Pri	3	NL	17	52.50	53.30	23.40	17.70
Pri	3	NL	18	26.00	34.10	19.40	17.60
Pri	3	NL	19	26.30	18.20	26.80	41.20
Pri	3	NL	20	29.90	26.30	19.10	22.20

<: readings were smaller than the number shown, but the computer screen could not accommodate the large indents and this was the smallest number it could register.



TABLE III

Statistical analysis of the Vicker's Hardness Test of reading #2 minus  
base line, reading #3 minus base line, and reading #3 minus reading #2

Type	L/NL	# Obs	Variable	#	Mean ppm	Std Dev	Std Error	Minimum	Maximum
Per	L	10	Diff 1	10	24.23	11.08	3.50	3.65	39.50
			Diff 2	10	-284.25	22.29	7.05	-312.33	-241.10
			Diff 3	10	-308.38	27.60	8.73	-333.68	-244.75
	NL	10	Diff 1	10	-1.37	9.32	2.95	-16.70	11.80
			Diff 2	10	-312.80	22.93	7.25	-336.00	-275.95
			Diff 3	10	-311.44	20.98	6.63	-335.35	-274.70
Pri	L	10	Diff 1	10	-7.66	13.02	4.12	-27.00	18.80
			Diff 2	10	-289.99	42.77	13.52	-334.88	-181.15
			Diff 3	10	-282.33	40.85	12.92	-318.58	-178.45
	NL	10	Diff 1	10	2.93	10.48	3.31	-22.95	12.15
			Diff 2	10	-259.58	43.01	13.60	-304.85	-173.53
			Diff 3	10	-262.50	40.17	12.70	-312.80	-185.68



TABLE IIIA								
The individual calculations of each chip for reading #2 minus base line, reading #3 minus base line, and reading #3 minus reading #2.								
Type	Lased	Chip	VHN Base	VHN 1	VHN 2	Diff 1	Diff 2	Diff 3
Per	L	1	339.40	368.05	43.10	28.65	-296.30	-324.95
Per	L	2	347.55	368.90	35.23	2.14	-312.33	-333.68
Per	L	3	343.65	377.80	52.28	34.15	-291.38	-325.53
Per	L	4	335.05	374.55	60.35	39.50	-274.70	-314.20
Per	L	5	342.05	345.70	100.95	3.65	-241.10	-244.75
Per	L	6	322.00	358.05	32.30	36.05	-289.70	-325.75
Per	L	7	347.10	364.80	48.43	17.70	-298.68	-316.38
Per	L	8	330.00	353.25	33.03	23.25	-296.98	-320.23
Per	L	9	331.75	357.25	80.78	25.50	-250.98	-276.48
Per	L	10	359.95	372.45	70.55	12.50	-289.40	-301.90
Per	NL	11	358.95	355.50	25.08	-3.45	-333.88	-330.43
Per	NL	12	357.60	356.10	72.48	-1.50	-285.13	-283.63
Per	NL	13	362.35	345.65	27.80	-16.70	-334.55	-317.85
Per	NL	14	340.10	351.10	33.98	11.00	-306.13	-317.13
Per	NL	15	348.70	360.50	54.50	11.80	-294.20	-306.00
Per	NL	16	356.00	362.55	27.20	6.55	-328.80	-335.35
Per	NL	17	351.25	350.25	19.13	-1.00	-332.13	-331.13
Per	NL	18	356.50	343.80	20.50	-12.70	-336.00	-323.30
Per	NL	19	344.35	343.10	68.40	-1.25	-275.95	-274.70
Per	NL	20	348.60	342.20	47.35	-6.40	-301.25	-294.85
Pri	L	21	340.60	336.70	27.48	-3.90	-313.13	-309.23
Pri	L	22	325.50	316.75	33.53	-8.75	-291.98	-283.23
Pri	L	23	361.10	335.60	26.23	-25.50	-334.88	-309.38
Pri	L	24	316.80	335.60	17.03	18.80	-299.78	-318.58
Pri	L	25	325.00	322.30	143.85	-2.70	-181.15	178.45
Pri	L	26	349.20	336.05	31.50	-13.15	-317.70	-304.55
Pri	L	27	326.90	326.70	23.50	-0.20	-303.40	-303.20
Pri	L	28	336.95	331.60	64.13	-5.35	-272.83	-267.48
Pri	L	29	312.50	303.65	38.80	-8.85	-273.70	-264.85
Pri	L	30	345.25	318.25	33.85	-27.00	-311.40	-284.40
Pri	NL	31	284.70	292.85	81.88	8.15	-202.83	-210.98
Pri	NL	32	301.70	313.85	128.18	12.15	-173.53	-185.68
Pri	NL	33	330.15	338.10	25.30	7.95	-304.85	-312.80
Pri	NL	34	322.00	299.05	25.98	-22.95	-296.03	-273.08
Pri	NL	35	322.35	333.95	45.80	11.60	-276.55	288.15
Pri	NL	36	308.60	310.90	66.15	2.30	-242.45	-244.75
Pri	NL	37	288.60	287.10	36.73	-1.50	-251.88	-250.38
Pri	NL	38	309.30	320.50	24.28	11.20	-285.03	-296.23
Pri	NL	39	295.10	296.50	28.13	1.40	-266.98	-268.38
Pri	NL	40	320.05	319.00	24.38	-1.05	-295.68	-294.63



<p>TABLE IV</p> <p>Statistical analysis of the confocal observations on all lased (L) and non-lased (NL), permanent (Per) and primary (Pri) chips.</p>									
Type	L/NL	# Obs	Variable	#	Mean ppm	Std Dev	Std Error	Minimum	Maximum
Per	NL	10	Confocal	10	6057.53	1448.37	458.02	3856.85	8348.15
Per	L	10	Confocal	10	6503.28	725.22	229.33	5450.85	7615.60
Pri	L	10	Confocal	10	7073.60	1625.83	514.13	4161.65	9302.65
Pri	NL	10	Confocal	10	8459.34	2737.07	865.54	2354.75	11205.50



<p style="text-align: center;"><b>TABLE IVA</b>  <b>The individual readings of all areas,</b>  <b>left and right, on all lased (L) and</b>  <b>non-lased (NL), permanent (Per)</b>  <b>and primary (Pri) chips.</b></p>					
#	L/NL	Per/Pri	Left	Right	Average
1	L	Per	7828.90	4678.10	6253.50
2	L	Per	7266.50	5314.00	6290.25
3	L	Per	7969.10	5231.60	6600.35
4	L	Per	7391.10	6324.90	6858.00
5	L	Per	6073.90	8425.80	7249.85
6	L	Per	5590.80	5310.90	5450.85
7	L	Per	6120.40	8171.70	7164.05
8	L	Per	6757.60	8473.60	7615.60
9	L	Per	5319.70	6779.60	6049.65
10	L	Per	5877.00	5160.50	5518.75
11	NL	Per	4679.30	5499.60	5089.45
12	NL	Per	4968.70	5639.90	5304.30
13	NL	Per	7590.50	8657.90	8124.20
14	NL	Per	7362.10	6004.70	6683.40
15	NL	Per	4643.50	6427.40	5535.45
16	NL	Per	3645.20	4068.50	3856.85
17	NL	Per	8325.20	8371.10	8348.15
18	NL	Per	8246.50	5524.10	6885.30
19	NL	Per	6429.30	5527.90	5978.60
20	NL	Per	4409.50	5129.70	4769.60
1	L	Pri	10610.00	5582.60	8096.30
2	L	Pri	7075.90	7957.20	7516.55
3	L	Pri	6797.90	5874.50	6336.20
4	L	Pri	6277.10	7816.90	7047.00
5	L	Pri	2587.20	5736.10	4161.65
6	L	Pri	3635.10	5668.80	4651.95
7	L	Pri	7235.70	8526.50	7881.10
8	L	Pri	6314.80	8189.90	7252.35
9	L	Pri	9479.40	9125.90	9302.65
10	L	Pri	7915.00	9065.50	8490.25
11	NL	Pri	0.00	4709.50	2354.75
12	NL	Pri	5375.00	6568.70	5971.85
13	NL	Pri	7954.50	9182.30	8568.40
14	NL	Pri	9570.00	12646.00	11108.00
15	NL	Pri	7781.30	6123.30	6952.30
16	NL	Pri	8084.20	8857.70	8470.95
17	NL	Pri	8353.80	12088.00	10220.90
18	NL	Pri	8878.50	10561.00	9719.75
19	NL	Pri	11964.00	8077.90	10020.95
20	NL	Pri	11616.00	10795.00	11205.50



FIGURE 1. Endo Technic laser - 35.



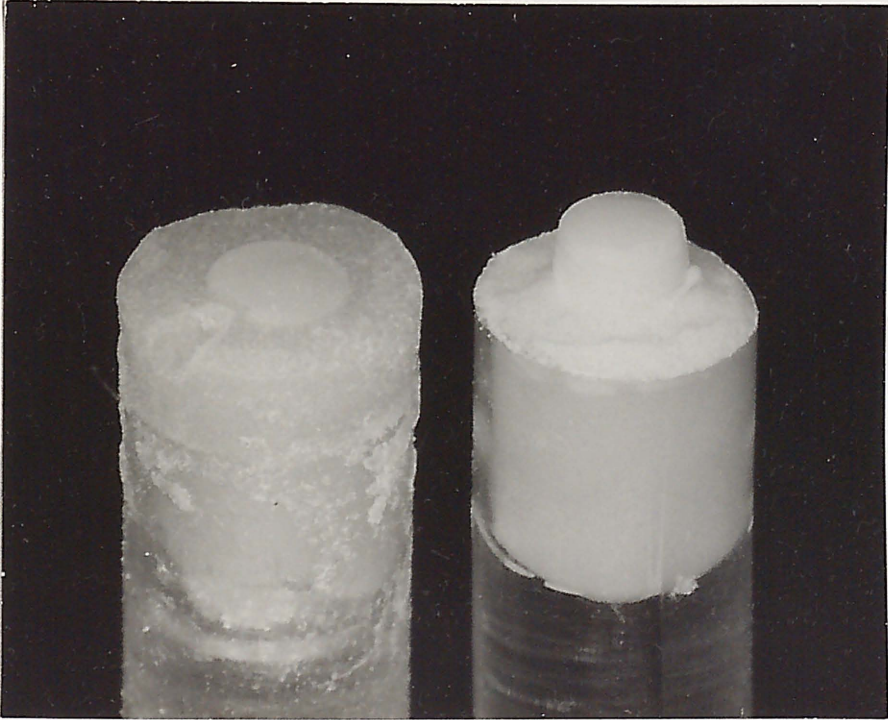
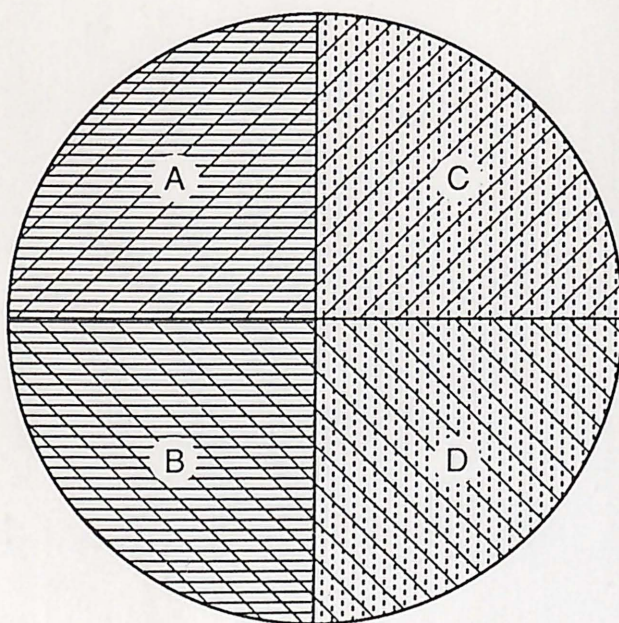


FIGURE 2. Mounted 3 mm enamel chip on a lucite rod.



FIGURE 3. Contact handpiece of the Nd:YAG laser.





- A – Acid demineralized enamel
- B – Sound covered enamel
- C – Lased acid demineralized enamel
- D – Lased covered enamel

FIGURE 4. Diagram illustrating the four different areas on the enamel chip for the SEM study.



FIGURE 5. 3 mm round diamond cutting cylinder, a part of the drill press machine.

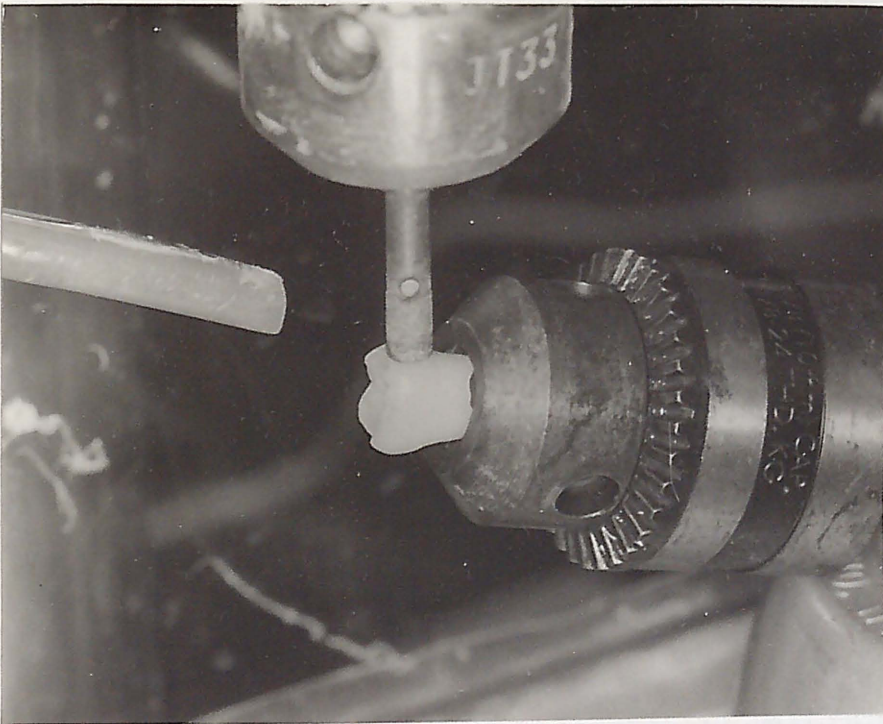


FIGURE 6. Drill press machine used to produce 3 mm chips of tooth structure.



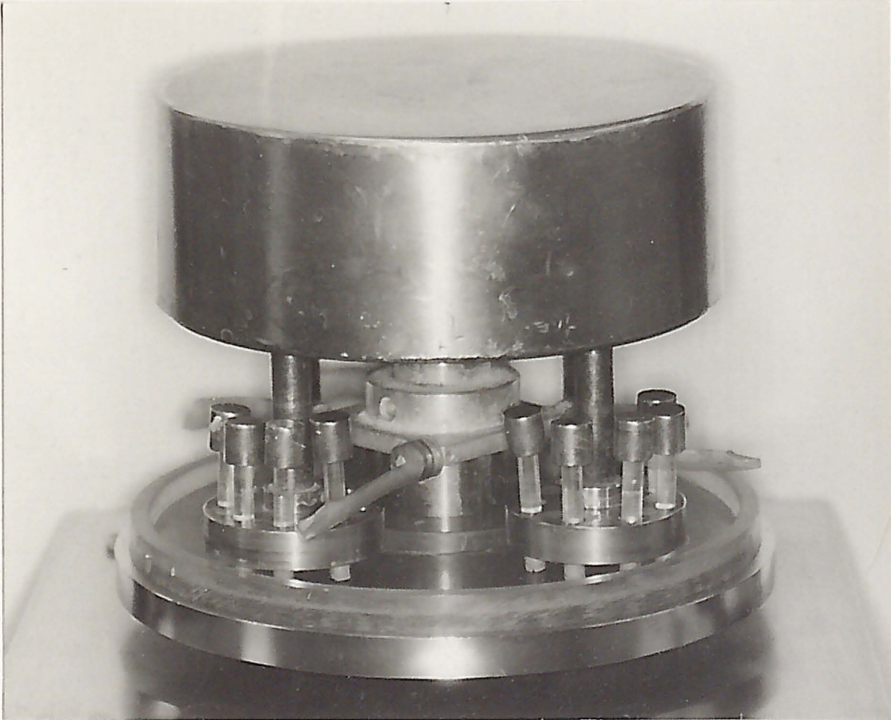


FIGURE 7. Set up for grinding and polishing the enamel chips.

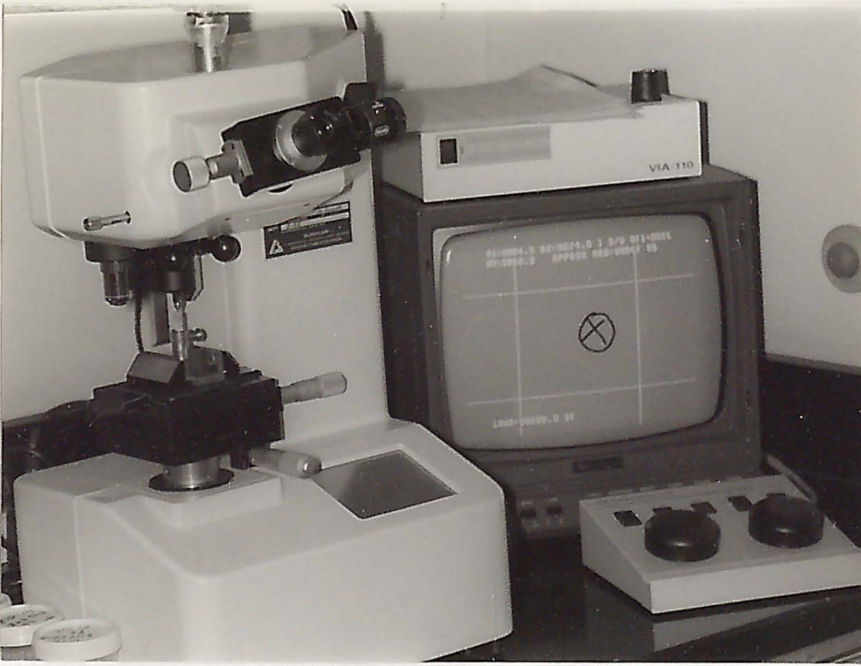


FIGURE 8. Set up for the Vicker's Hardness Test.

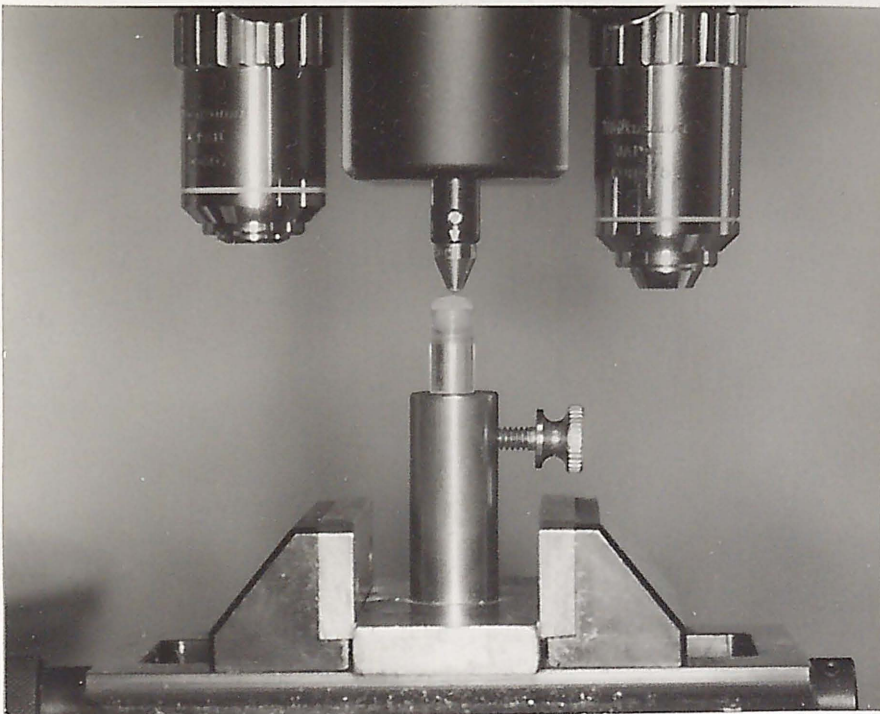


FIGURE 9. Placing the 200 g load for the hardness test.





FIGURE 10. Applying the 200 g load on the enamel chip.

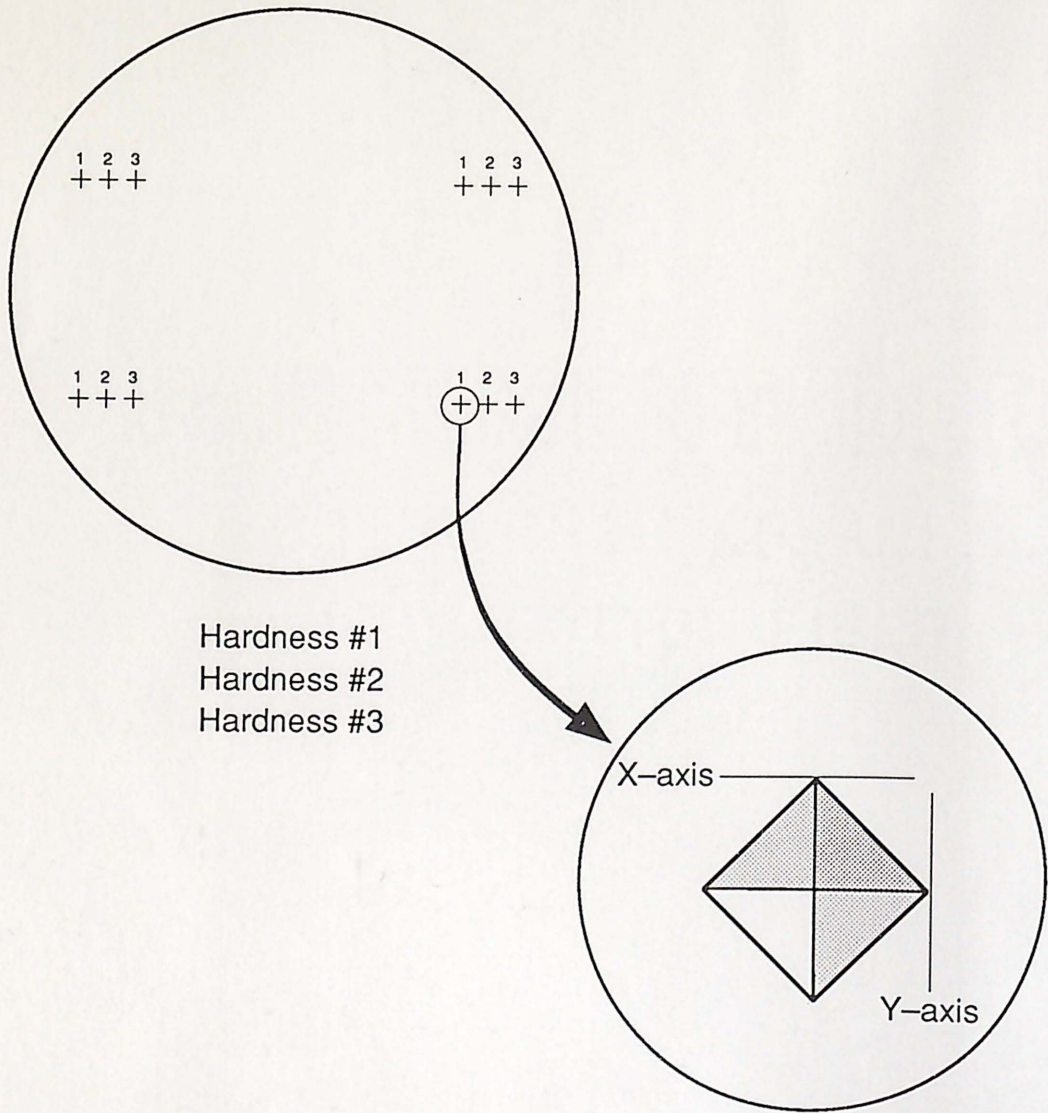


FIGURE 11. Diagram indicating the areas where the hardness tests were done and computed.



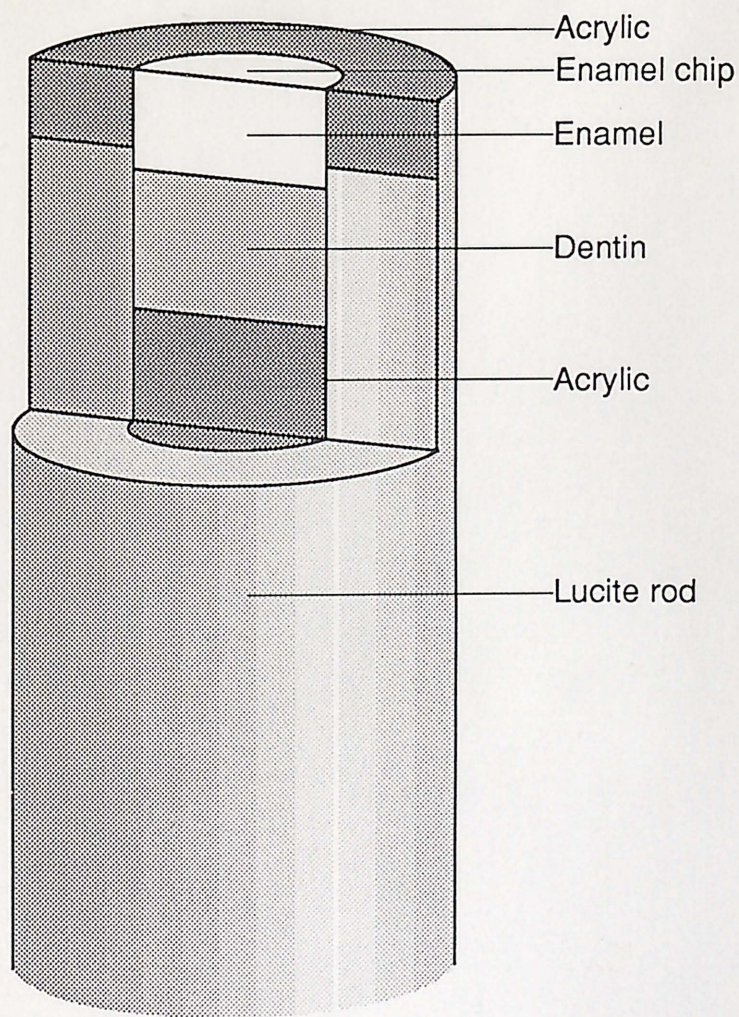


FIGURE 12. Diagram showing the cross section of the enamel chip and the lucite rod.



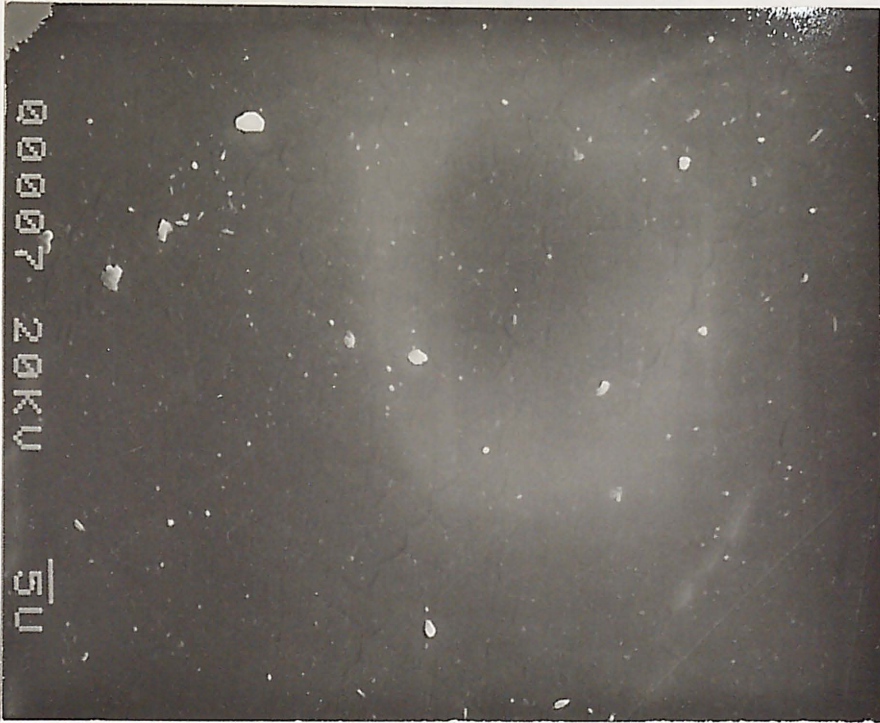


FIGURE 13. SEM: Primary smooth surface, non-lased/non-etched.

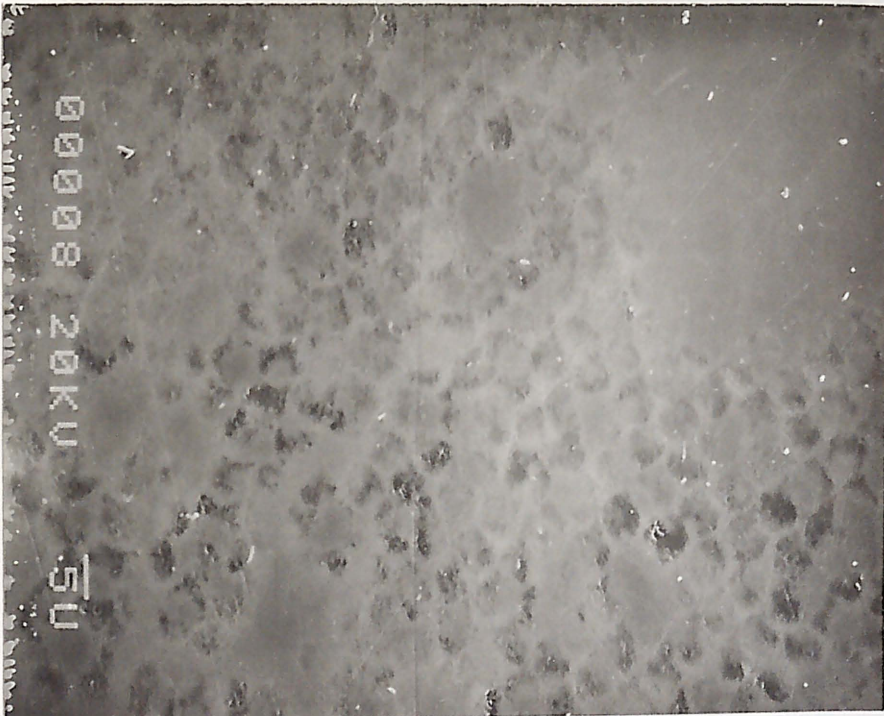


FIGURE 14. SEM: Primary smooth surface, non-lased and acid etched. Enamel prisms with smooth surface indicated.



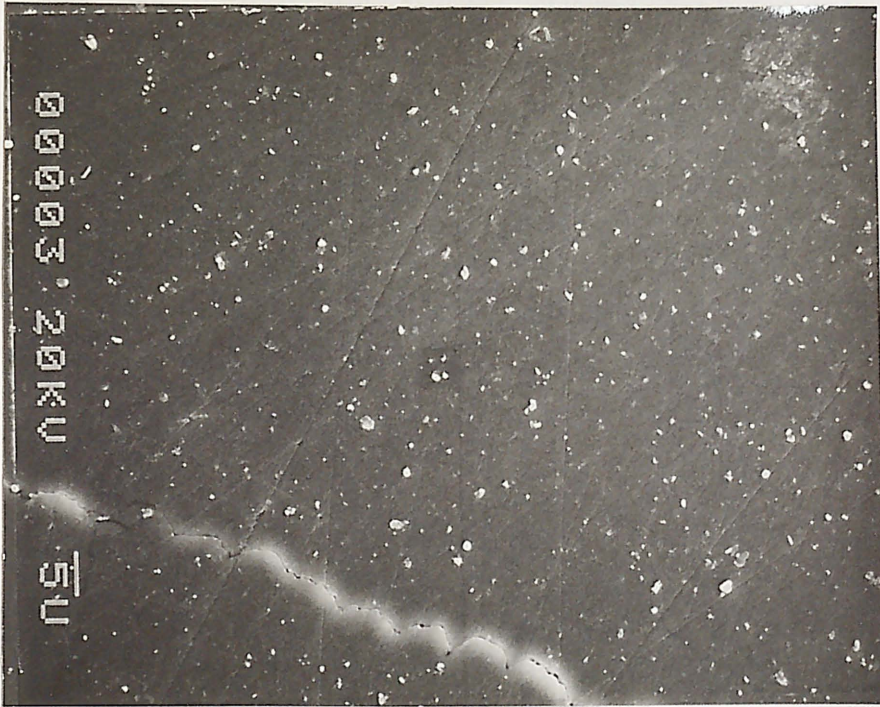


FIGURE 15. SEM: Permanent smooth surface, lased and acid etched. Debris and scratches seen.



FIGURE 16. SEM: Permanent smooth surface, lased and non-etched. Smooth surface with debris and scratches noted.





FIGURE 17.

SEM: Primary smooth surface, specimen #4.  
Scratches were seen in all four areas.





FIGURE 18. SEM: Permanent pit and fissure, lased/acid etched, cracking.



FIGURE 19. SEM: Permanent, pit and fissure, non-lased and etched. Enamel prisms.



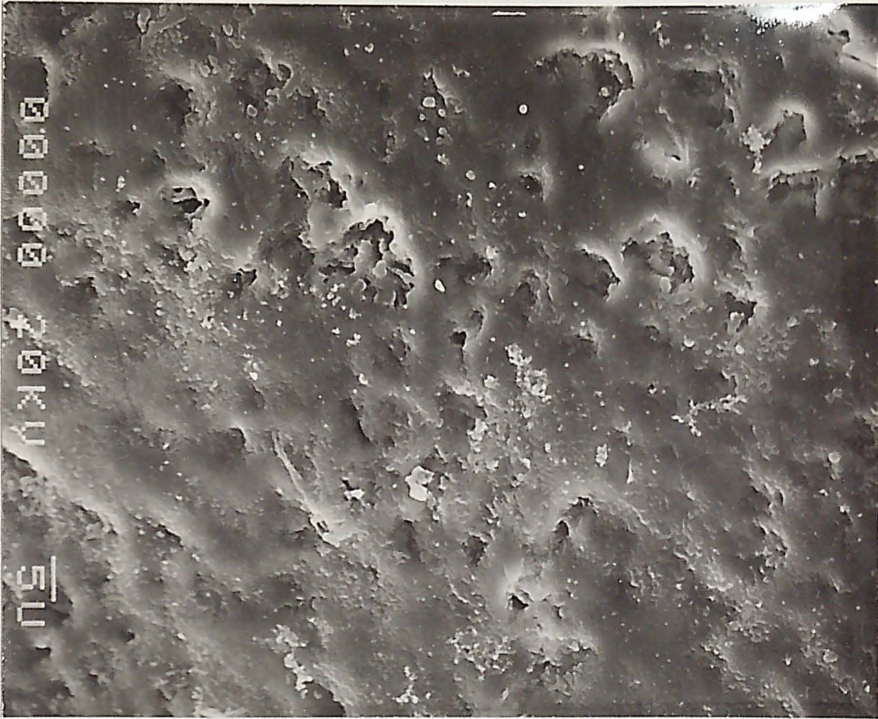


FIGURE 20. SEM: Permanent, pit and fissure, lased and non-etched, cratering.

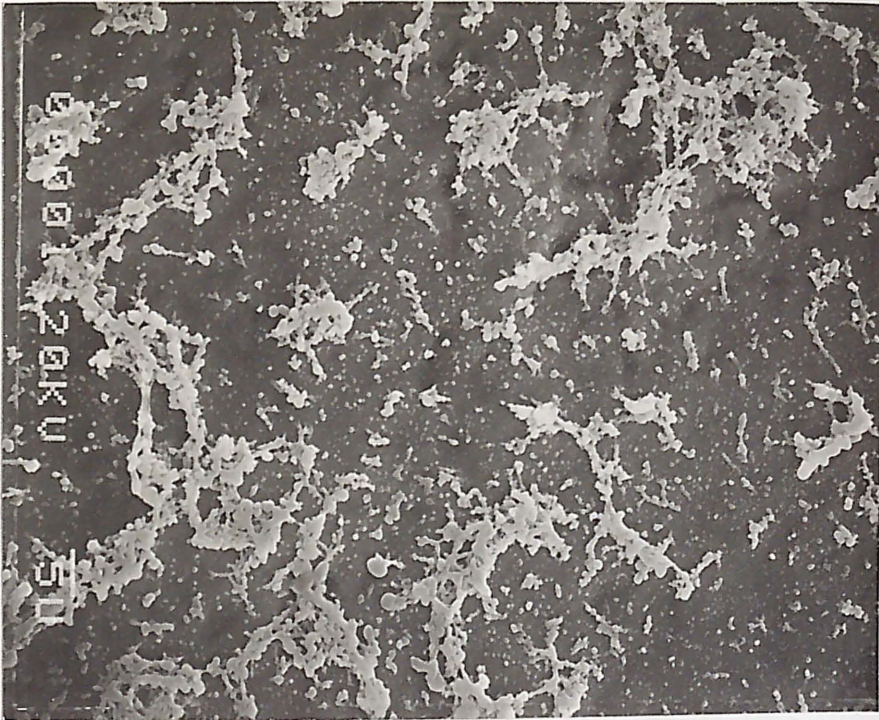


FIGURE 21. SEM: Permanent, pit and fissure, lased and acid etched, white bubbles and cobblestone appearance.



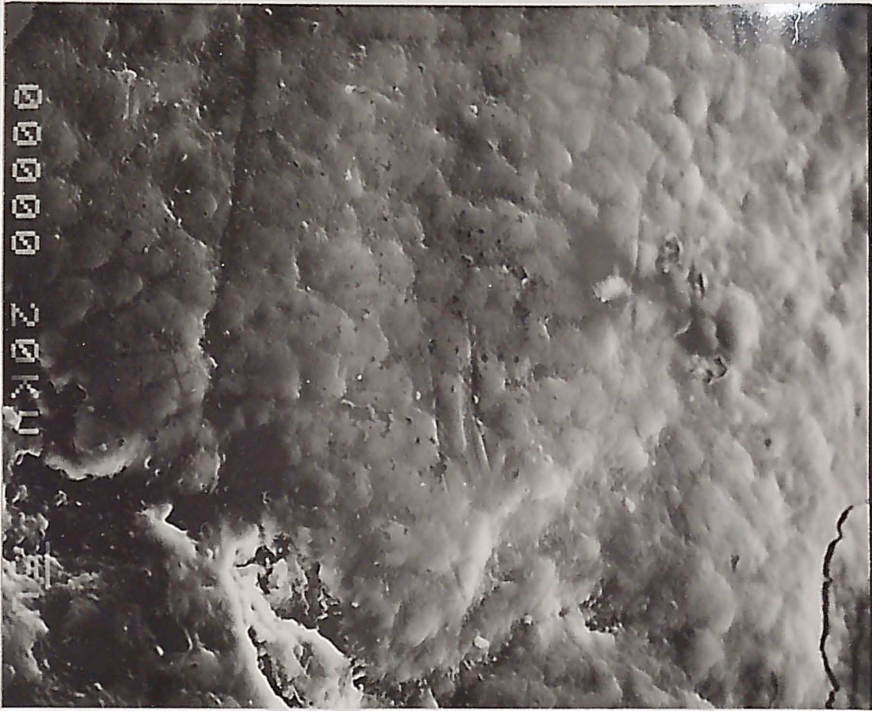


FIGURE 22. SEM: Primary, pit and fissure, lased and non-etched. Rough surface.



FIGURE 23. SEM: Primary, pit and fissure, non-lased and non-etched, ridges.





FIGURE 24. SEM: Primary, pit and fissure, lased and non-etched, ravines.

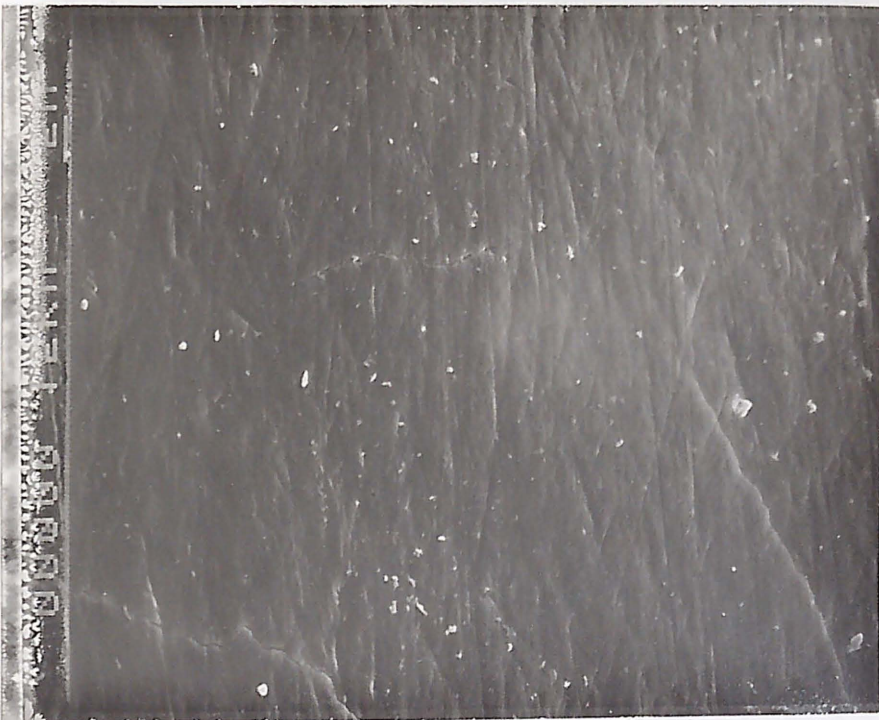


FIGURE 25. SEM: Primary, pit and fissure, lased and acid etched, smooth surface.



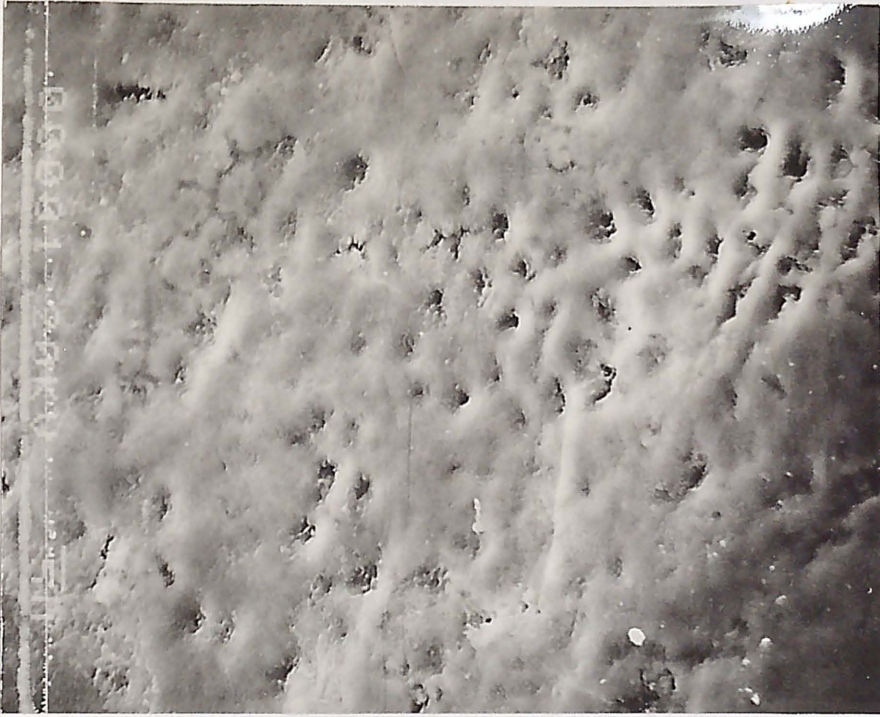


FIGURE 26. SEM: Permanent, pit and fissure, lased and non-etched, cratering and pitting.

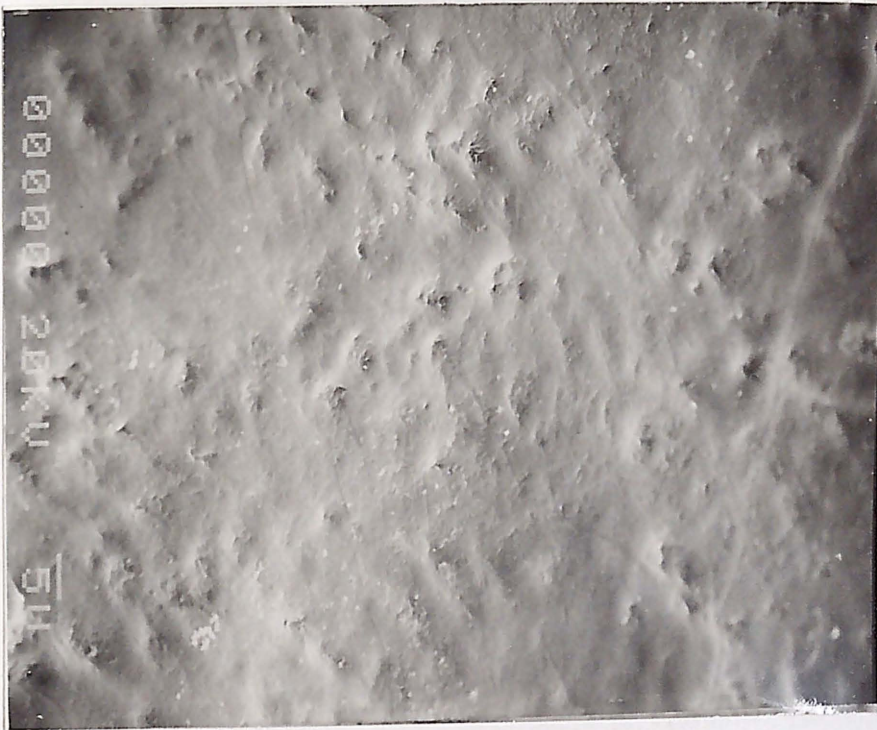


FIGURE 27. SEM: Permanent, pit and fissure, lased and etched, cratering and pitting.





FIGURE 28. Confocal, primary lased, 250 micron area of demineralized enamel of 6600.35 square microns.

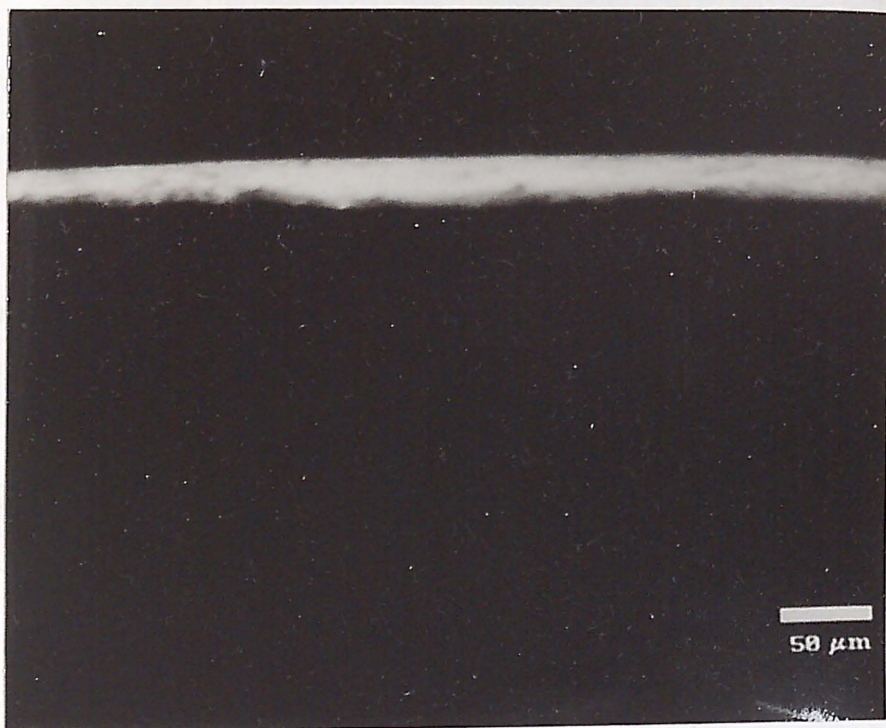


FIGURE 29. Confocal, primary non lased 250 micron area of demineralized enamel of 8124.40 square microns.





FIGURE 30. Confocal, permanent lased, 250 micron area of demineralized enamel of 6253.50 square microns.

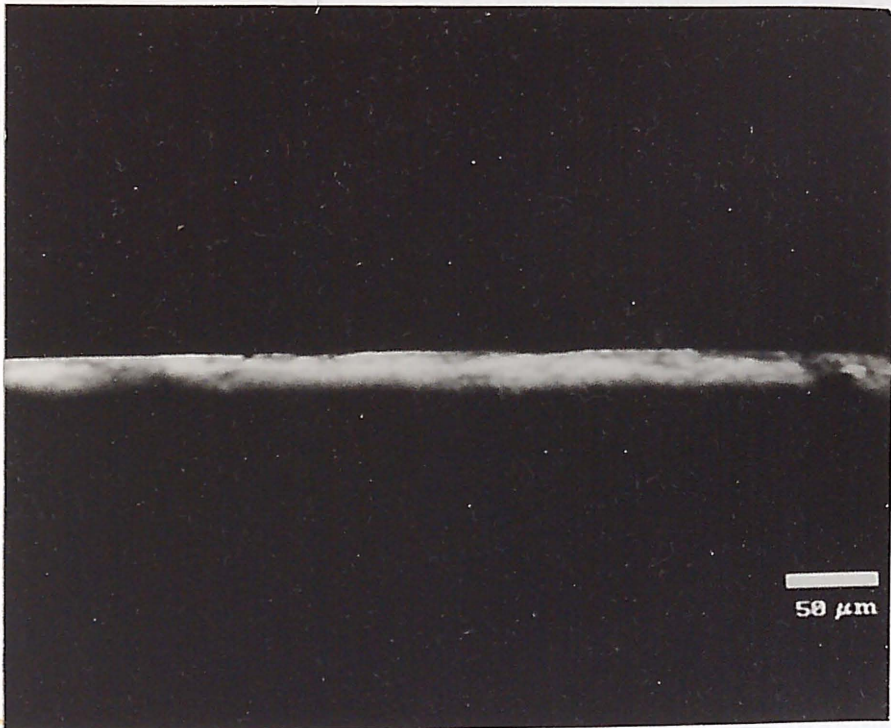


FIGURE 31. Confocal, permanent non-lased 250 micron area of enamel of 5304.30 square microns.

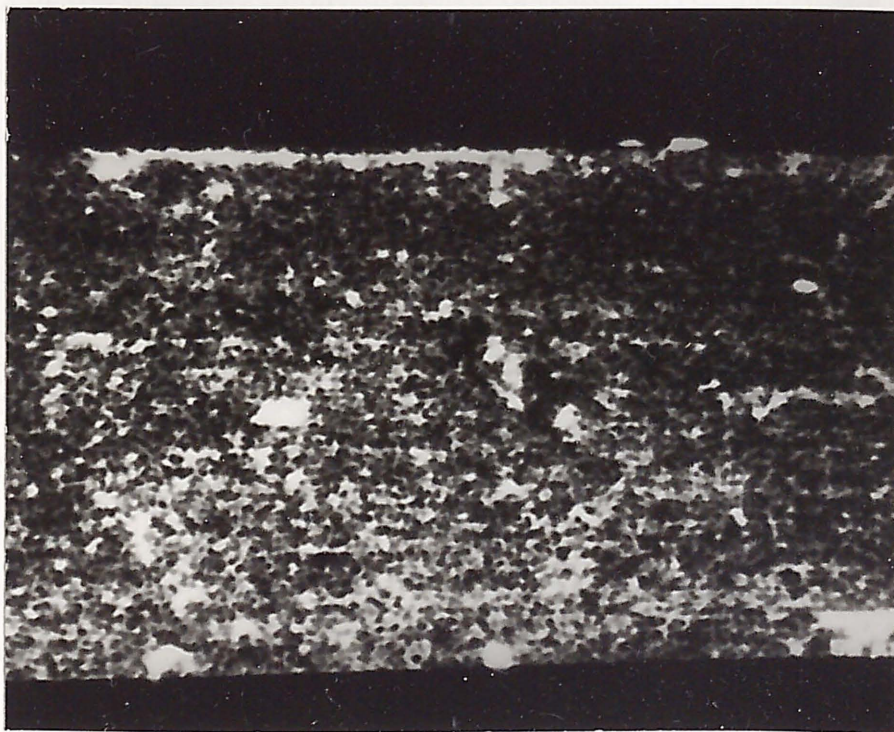


FIGURE 32. Confocal, control, no area of demineralized enamel for all groups.



## DISCUSSION

### Pilot Study

The results that were seen on the SEM with the different laser power settings were of no help to this research project. There were too many variations and no consistent findings for a specific power. In addition, significant alterations and damage occurred such as pitting, cratering and cracking. Therefore, following personal communication with the laser manufacturer, a power setting for all the tests of 3 watts was decided upon. Note that this is the lowest power setting possible for the particular laser machine used in this study.

One note of interest during the pilot study was that the primary teeth became warm to the touch a lot faster than the permanent teeth. The permanent teeth were generally complete teeth that had not sectioned at the roots, while the primary teeth roots for the most part were resorbed. The higher the energy used the warmer the tooth became.

### Parameter #1-SEM Evaluation

The SEM results that were obtained for the study were designed to be analyzed using a Fisher's exact test to compare the distribution of damaged vs. non-damaged and lased vs. non-lased specimens. Since the results that were obtained did not have a specific pattern (see Appendixes X and XI), the quantitative analysis was eliminated, and a qualitative analysis was used instead.



The results expected for this study, based on the work of previous researchers,<sup>32</sup> were that ablated surface morphology from any laser medium should be a roughened texture similar to acid etched enamel and that enamel fusion of lased surfaces would be encountered.<sup>47</sup> Other observations expected were a crazed and mottled surface with white surface opacities.<sup>53</sup> A study using low power, between 2-5 watts, showed localized melting followed by recrystallization and little surface loss.<sup>44</sup> All these observations were seen in this study except for the melting. The results noted were seen both in the lased and non-lased enamel, which is not consistent with the previous research since these observations were only seen in the lased areas. For example, permanent specimen #4 had a cobble stone appearance that was seen in the non-lased, non-etched area (Appendix X). This is a feature that should only be seen in the lased areas.

All smooth surface chips had a surface with apparently no surface change characteristics. They were all very smooth except for one specimen, which was primary chip #4. The reason for these findings is most likely due to the polishing technique used. By using the Gamma micropolish Alumina B #3 particle, a smear layer might have formed that cannot be washed with distilled water, and therefore, the appearance seen in the SEM was very smooth. The electrons could not penetrate that first layer and so no difference was detected. As for specimen #4, the reason that the surface was so rough was because it might not have been polished accurately. The surface showed a lot of scratches that might have been created by the 600 micron grit of carbamet paper disc (see Figures 13-17).



There are no previous studies using SEM that have used this polishing compound to compare with the current results. The compound is used to create a nice finished polished surface of enamel after grinding. Possibly this step can be eliminated for the SEM to obtain more accurate results. The reason it was used in this study was to maintain consistency within the specimens.

As for the pit and fissure specimens that were not polished, a pattern was noticed for the first three specimens. Craters were formed with lasing, and after etching the craters became shallower. This could be due to the acid etching reversing what the laser had created. The etching in this experiment did follow the lasing. The craters might have existed initially, the laser might have coalesced them and made them shallower, and the etching might have overridden the lasing effect (see Figures 26-27). No difference was observed between the permanent and primary teeth.

#### Parameter #2 - Enamel Acid Resistance

The analysis of calcium and phosphorous was conducted to determine if there was a difference between the primary and permanent teeth in their dissolution rate, especially after laser use. It was expected that the primary teeth would have more of a dissolution rate than the permanent due to the difference in organic vs. inorganic content. As stated previously, the organic content of primary teeth is between 7-8% and for permanent teeth it is between 4-5%, making primary enamel more soluble in acid solutions.



The phosphorous analysis was as expected. More phosphorous ions were detected in the primary than the permanent teeth in all 20 specimens in both groups. The average was 85.15 ppm in primary compared to 53.15 ppm in permanent teeth, which was a significant difference ( $p=0.0001$ ). As for the calcium ions, it was similar to that of phosphorous where the primary teeth were more soluble with an average of 2.62 ppm and the permanent with 1.83 ppm. This included all 40 specimens, which was also significantly different ( $p=0.0001$ ).

As was noted earlier in previous research,<sup>34</sup> the dissolution rate of lased enamel under conditions corresponding to a modest acid attack is expected to be much less than that of unlased enamel. Using a laser in this study made the teeth less soluble to acid dissolution and this was noted for both the calcium and phosphorous, but it varied with the tooth type. For the phosphorous ions both the permanent and primary lased specimens acted the same in that both decreased in solubility compared to the non-lased control group. There was no variation between the two. The average for all 20 permanent and primary lased chips was 65.2 ppm and the 20 non-lased chips average was 73.1 ppm. This was statistically different ( $p=0.0111$ ). The interaction between the type of tooth and L/NL was not significant ( $p=0.6326$ ). Therefore, the primary teeth and the permanent teeth behaved similarly with laser use.

The results for the calcium dissolution was a bit different. The interaction between the type of tooth and L/NL was significant ( $p=0.0126$ ). In the permanent teeth there was no difference between the lased and non-lased chips where the average for the

lased was 1.81 ppm and the non-lased was 1.85 ppm, which contradicts the assumption that there would be a difference between lased and non-lased. In the primary teeth there was a difference between the lased and non-lased where the average was 2.34 ppm for (L) and 2.90 ppm for the (NL), with a significant difference ( $p=0.0126$ ), where the primary lased teeth had reacted differently than permanent lased teeth and the expected would be the opposite, where both types would react in a similar fashion.

The results here generally coincided with Yamamoto and Ooya<sup>30</sup> in that the greatest degree of demineralization occurred with the unlased normal enamel surface while the least demineralization was seen with the lased enamel. In this case, the laser affected the primary teeth more than the permanent teeth. It has been shown in previous research that depending on the localized tooth enamel temperature produced by laser-irradiant conditions (energy density and the absorption coefficient of tooth enamel for the laser light wavelength employed) and, in part, water vapor pressure, various compositional, structural, and phase changes will occur.<sup>31</sup> Therefore, small variations in tooth color and opacity could be responsible for some of the variations seen.<sup>53</sup> Primary teeth are usually lighter in color than permanent teeth.<sup>54</sup>

The energy used in this study was low, and in some studies where low energy was used<sup>32</sup> no difference was seen in the dissolution and solubility. The results for the calcium ions can be an indication that the laser was strong enough for the primary teeth to cause a difference. As for the permanent teeth, a stronger energy was needed to cause the same difference. Changes in mineral



distribution can occur with laser exposure. Primary and permanent teeth differ in their mineral content as described earlier. Due to this fact, the calcium and phosphorous in the two types of teeth can act differently with laser exposure. The concentration of calcium and phosphorous are altered after laser exposure, where a higher Ca/P ratio is seen due to the decreased phosphorous content after lasing.<sup>29</sup> This explains why the study showed more phosphorous ions than calcium ions in the acid solution. The contents of water, carbonate and organic substances are reduced in lased enamel,<sup>2</sup> and since primary teeth have more organic material than permanent, the effects of the laser is observed more on primary than permanent teeth.

### Parameter #3- Vicker's Hardness Test

The microhardness test was designed to determine if lased enamel becomes harder than that of non-lased. Since the laser made the enamel more resistant to acid etching, it is assumed that the tooth becomes harder when exposed to the laser. Results for the hardness test showed that permanent teeth were harder than primary teeth at baseline, and this is due to the difference in composition between the two types. The average hardness for the permanent was 346.1 VHN, and the average for the primary was 321.1 VHN, which was a significant difference ( $p= 0.0001$ ).

The hardness for the lased teeth was significantly higher than for the non-lased teeth. The average hardness for the lased was 345.2 VHN and for the non-lased was 331.1 VHN, which is



significantly different ( $p=0.0007$ ). This was the same for both the permanent and primary teeth.

The third reading was done after attacking the chips with carbopol acid solution for 48 hours. The permanent and primary teeth, lased and non-lased, acted similarly. The hardness readings were very low, meaning that the tooth had become softer. The average for the permanent was 47.7 VHN and for the primary was 46.3 VHN ( $p=0.4960$ ) that is not significant. The average for the lased was 49.8 VHN, and for the non-lased it was 44.2 VHN ( $p=0.4476$ ) which is also insignificant.

Since there was some difference in the initial readings, a decision was made to statistically take the difference from the baseline reading and see if that changed the results. In the analysis of the difference of reading 2 from baseline, where reading 2 was done after the chips were exposed to the laser only, the type of tooth and lased/non-lased combination had a significant difference ( $p=0.0001$ ). The lased permanent teeth increased in hardness 24.23 VHN compared to the non-lased permanent teeth. Whereas, the lased primary teeth decreased 7.66 VHN compared to the non-lased primary teeth. This means that the primary teeth had become softer after exposure to the laser. This result coincided with the study of Ferreira where he showed that lased enamel was softer than unlased enamel.<sup>36</sup> This was only seen in primary teeth which could be for the same reason as mentioned above. Possibly the energy used here was strong enough to affect the primary teeth but not strong enough to affect permanent teeth to change any of its ultrastructure. The reason given by Ferreira for softer enamel was observed by the use



of Transmission Electron Microscopy. Exposed enamel revealed that most crystals generally resembled those of unlased enamel in size and shape, but inter- and intra-crystalline voids were present in some areas of the lased surface. He observed that the enamel showed significant ultrastructural changes; new homogeneous and inhomogeneous crystals of apatite with a different shape and larger size than those of the original, and a loss of prismatic structure was seen.<sup>36</sup>

For the non-lased teeth, the difference from the baseline was not significantly different between permanent and primary teeth since no alteration was done on the specimens. The average hardness for the permanent decreased by 1.37 VHN and increased for the primary by 2.93 VHN. This small variation could be due to the variations seen from area to area and sample to sample<sup>36</sup> while conducting the hardness test. This is why the punches taken were made in such close proximity from one to another.

The analysis of the difference in reading 3 from the baseline, (where reading 3 was done after the chips were exposed to the laser and immersed in Carbopol acid solution), showed that the interaction of the type of tooth and L/NL had a significant effect on the difference between the reading and the baseline ( $p=0.0023$ ). The lased teeth did not differ between the primary and the permanent. However, the non-lased permanent teeth had a significantly larger difference from the baseline than the primary teeth. The average hardness for the primary teeth decreased in reading 3 just as the permanent, but not to the same degree. The average difference for



the permanent decreased by 312.8 VHN and the primary decreased by 259.6 VHN.

The permanent non-lased teeth had a larger difference than lased teeth. This shows that the permanent teeth did get harder with lasing and so the demineralization in the acid solution was less for the lased. This observation correlates with the other studies. As for the primary teeth, the lased teeth had a larger difference from the baseline than the non-lased teeth, which is opposite to what was expected. The only explanation that can be given here is that these results coincide with Ferreira's study where the lased enamel became softer<sup>36</sup> and demineralized more making the difference between this reading and the baseline larger.

Another comparison was done where the difference between the third and the second reading were taken. The type of tooth made a difference on the average. The permanent teeth difference was decreased by 309.0 VHN and the primary teeth difference was decreased by 272.4 VHN. The L/NL variation did not have a difference. The reason for this could be the assumption that the permanent started out harder, in general, than the primary, but when it was acid challenged it became softer. When it reached a certain softness the type of tooth did not matter any more and probably the rate of dissolution became similar for both the permanent and primary teeth. Therefore, if the primary teeth started out softer, they had only to decrease a certain amount to reach this softness, which makes the difference look smaller compared to the permanent teeth.



#### Parameter #4-Caries-like Lesion Formation

For the confocal results, an average of two readings was taken. The depth of the lesion created by submitting all the chips to an acid challenge was calculated by looking at a 250 micron area. To make sure that these lesions were created after the acid challenge, a control group not submitted to acid attack of six primary chips, three lased and three unlased, were observed. There was no decalcification in any area (see Figure 32). A similar procedure was also conducted on six permanent chips. This showed that the lesions observed on the confocal were actually created by the acid challenge after the chips had been lased.

The results show that the effects of the type of tooth made a difference, since the lesions created in the primary teeth were deeper than those created in the permanent teeth. The primary average area of demineralized enamel was 7,766.5 square microns, and the average for the permanent was 6,280.4 square microns. This was a significant difference ( $p=0.0124$ ). These results were expected since the primary teeth consist of more organic material that makes it more soluble in acid solution. The lased and non-lased relation did not have a significant effect ( $p=0.4108$ ). The average for the lased teeth was 6,788.4 square microns and the average for the non-lased was 7,258.4 square microns (see Figures 28-31).

Previous research has shown that the exposure of teeth to laser irradiation made them more resistant to acid dissolution. In this portion of the study, the lased teeth did not show any difference in acid resistance. This could be due to the time utilized to do the acid dissolution. It could have been that 48 hours in the Carbopol



solution was too long. Forty-eight hours were used here to give a typical lesion on sound enamel and was a closer simulation of intra oral environment. Fox, et al.<sup>34</sup> have indicated that after ten minutes, the dissolution rate for the lased enamel had increased and become equal to the unlased enamel. This showed that the mineral that was affected in the top layer had dissolved, and so the remaining uncovered enamel behaved similarly to unlased enamel. In this study, this might have occurred such that the layer that was affected by the laser was dissolved by the Carbopol and then the lased chips acted in the same manner as the unlased chips.

### Final Discussion

This study was conducted to determine any differences between the behavior of permanent enamel to primary enamel after it has been exposed to laser irradiation. There were many challenges that were faced, and the biggest one was the variation that existed between the teeth and within the actual chips. As Brudevold and Soremark<sup>3</sup> commented, there are real and sometimes marked differences in the composition of enamel mineral. Enamel from individual teeth of a single person can vary in composition as much as that of teeth from different persons.

A difference between the permanent and primary teeth was expected before the conduction of this study. The observations from other studies have stated that changes occur from lasing teeth depending on wavelengths, power outputs, energy densities, duration of exposure, characteristic absorption of the particular tissue and its prismatic structure and its orientation. Since the primary teeth are



lighter in color than permanent teeth, a difference should be seen. In primary teeth the prisms have no perikymata. There are many different directions of the prisms in relation to the enamel surface,<sup>3</sup> all these differences can lead to a change in the effect of the laser. Another difference between the permanent and primary teeth is crown and root morphology. Primary teeth have a thinner layer of enamel that covers the dentin and the pulp, and relatively larger pulps as well as more pronounced pulp horns. Therefore, caution should be taken when a laser is used with primary teeth.

Different factors could have been changed in this study. A more powerful energy level could have been used to create more prominent differences. The time in the Carbopol could have been decreased where a difference between the lased and non-lased might have been seen. The polishing step should be eliminated if the chips are to be observed under the Scanning Electron Microscope, since none of the chips turned out. The use of India Ink has been noted to help with the use of the Nd:YAG laser. It was found that the blackness of the ink would absorb the laser beam at the enamel surface and prevent the beam from penetrating excessively into the tooth structure.<sup>55</sup> This might allow the use of a laser with a higher energy and not create as much damage for the rest of the tooth structure.

From the observations in this study, further evaluation and research is needed. Many studies have been done on permanent teeth, but more are needed for the primary teeth. It is clear from this study that the same recommendations cannot be used for both the primary and permanent teeth.

## SUMMARY AND CONCLUSIONS



The purpose of this study was to determine the effects of the Nd:YAG laser on the pits and fissures, and smooth surfaces of human primary enamel compared to the effects of the laser on permanent enamel. The hypothesis of the study was that Nd:YAG laser induced changes on the enamel of primary teeth are similar to the changes induced on the enamel of permanent teeth, with regard to acid solubility, resistance to caries-like lesion formation, surface topography and surface hardness.

Disassociated teeth were collected from pediatric dentists in the Indianapolis area and the department of oral surgery at Indiana University School of Dentistry. The primary teeth collected were either extracted for orthodontic reasons or exfoliated. The permanent teeth were extracted. All teeth were noncarious and nonrestored.

Three millimeter enamel chips were taken and polished to give a flattened surface. The laser equipment used was the Nd:YAG Endo Technic laser-35, which was provided by Laser Medical Technologies known as Biolaze. Four parameters were studied:

- 1) Scanning electron microscopic comparison of lased and non-lased enamel.
- 2) Enamel acid resistance as measured by calcium and phosphorous release.
- 3) Vicker's Hardness of the lased and non-lased enamel.



4) Confocal microscopic examination of caries-like lesion formation on lased and non-lased enamel.

The SEM study did not show a significant difference between the permanent and primary smooth and pit and fissure enamel. There were no certain qualities that could be identified in a specific area. Observations seen could not be quantified or qualified. This was true for the type of tooth and lased/non-lased relationship.

The analysis for calcium disassociation showed that the interaction between the type of tooth and lased/non-lased relationship was significant ( $p=0.0126$ ). The calcium disassociation was higher in primary teeth than permanent and only different in the lased primary but not in the lased permanent. Therefore, the lased primary enamel acted differently from that of lased permanent enamel. As for the phosphorous disassociation, there was no significant difference between the interaction of the type of tooth and the lased/non-lased relationship. Both permanent and primary teeth acted the same even though the phosphorus disassociation was generally higher for the primary enamel. In general, the lased teeth had a lower phosphorous release than the non-lased teeth.

The surface hardness test showed that the primary lased enamel became softer than the non-lased enamel. However, the permanent lased enamel became harder than the non-lased. In general the enamel was harder in all cases for the permanent teeth. When challenged with acid solution, both types of teeth and lased and non-lased enamel behaved similarly, in that their hardness scores were lower.



The Confocal Microscope observations showed no significant difference between the interaction of the type of tooth and lased/non-lased relationship. Both had formed decalcified lesions even though the lesion depth was deeper for the primary teeth in all cases.

In summary, this study showed several variations between lased permanent and primary teeth. It also showed a wide range of variations between the teeth and within the specimens themselves. Additional research on the effects of lasers on primary dental hard tissue is still needed.

## REFERENCES



1. Nelson DG, Shariati M, Glena R, Shields CP, Featherstone JD. Effect of pulsed low energy infrared laser irradiation on artificial caries-like lesion formation. *Caries Res* 1986;20:289-99.
2. Oho T, Morioko T. A possible mechanism of acquired acid resistance of human dental enamel by laser irradiation. *Caries Res* 1990;24:86-92.
3. Brudevold F, Soremark R. Chemistry of the mineral phase of enamel. In: Miles. Structural and chemical organization of teeth. New York: Academic press, 1967:247-77.
4. Miserendino LJ, Neiburger EJ, Pick RM. Current status of lasers in dentistry. *Illinois Dent J* 1987;56:254-7.
5. Garber DA. Dental lasers-myths, magic, and miracles? Part I: Introduction to laser in dentistry. *Compendium* 1991;12(7):448-52.
6. Miller M, Truthe T. Lasers in dentistry: an overview. *J Am Dent Assoc* 1993;124:32-5.
7. Dedrich DN. Laser/Tissue interaction: What happens to laser light when it strikes tissue? *J Am Dent Assoc* 1993;124:57-61.
8. Myers T. What lasers can do for dentistry and you. *Dental Manage* 1989;29(11):26-8,30.
9. Midda M, Renton Harper P. Lasers in dentistry. *Br Dent J* 1991;170(9):343-6.
10. Garber D. Dental lasers-myths, magic, and miracles? Part II: Present and future uses. *Compendium* 1991;2(7):698-708.
11. Pick RM. Using lasers in clinical dental practice. *J Am Dent Assoc* 1993;124:37-47.



12. Myers TD, McDaniel JD. The pulse Nd:YAG dental laser: Review of clinical applications. *Cal Dent Assoc* 1991;25-30.
13. Melcer J, Chaumette MT, Melcer F, et al. Treatment of dental decay by CO2 laser beam: Preliminary results. *Lasers Surg Med* 1984;4:311-21.
14. Myers TD, Myers WD. In vivo caries removal utilizing the Yag laser. *J Michigan Dent Assoc* 1985;67(2):66-9.
15. White J, Goodis H, Setcos J, Eaker S, Hulscher BE, Rose CL. Effects of pulsed Nd:YAG laser energy of human teeth: A three-year follow-up study. *J Am Dent Assoc* 1993;124:45-51.
16. Goodis HE, White JM, Harlan L. Absence of pulpal response from Nd:YAG laser exposure on enamel [Abstract]. *J Dent Res* 1992;71:162.
17. Goldman L, Gary JA, Goldman J, Goldman B, Meyer R. Effect of laser beam impacts on teeth. *J Am Dent Assoc* 1965;70:601-6.
18. Stern RH, Sognnaes RF. Laser beam effect on dental hard tissues. *J Dent Res* 1964;43:873.
19. Sognnaes RF, Stern RH. Laser effect on resistance of human dental enamel to demineralization in vitro. *J Southern Cal Dent Assoc* 1965;33:328.
20. Stern RH, Sognnaes RF Goodman F. Laser effect on in vitro enamel permeability and solubility. *J Am Dent Assoc* 1966;73:838-43.
21. Nelson DG, Jongebloed WL, Featherstone JD. Laser irradiation of human dental enamel and dentine. *NZ Dent J* 1986;82:74-7.
22. Vahl J. Electron microscopical and xray crystallographic investigations of teeth exposed to laser rays. *Caries Res* 1968;2:10-8.
23. Yamamoto H, Sato K. Prevention of dental caries by Acousto-optically Q switched Nd:YAG laser irradiation. *J Dent Res* 1980;59(2):137.



24. Yamamoto H, Sato K. Prevention of dental caries by Nd:YAG laser irradiation. *J of Dent Res* 1980;59:2171-7.
25. Nelson DG, Wefel JS, Jongebloed WL. Morphology, histology, and crystallography of human dental enamel treated with pulsed low energy infrared laser radiation. *Caries Res* 1987;21:411-26.
26. Hicks MJ, Flaitz CM, Westerman GH, Berg JH, Blankenau RL, Powell GL. Caries like initiation and progression in sound enamel following argon laser irradiation: An in vitro study. *J Dent for Children* 1993;60:201-6.
27. Kantola S, Laine E, Tarna T. Laser induced effects on tooth structure VI. Xray diffraction study of dental enamel exposed to a CO2 laser. *Acta Odontol Scand* 1973;31:369-79.
28. Kurado S, Fowler BO. Compositional, structural and phase changes in in vitro laser irradiated human tooth enamel. *Calcified Tissue International* 1984;36:361-9.
29. Kantola S. Laser-induced effects on tooth structure V Electron probe microanalysis and polarized light microscopy of dental enamel. *Acta Odontol Scand* 1972;30:475-84.
30. Yamamoto H, Ooya K. Potential of YAG laser in caries prevention. *J Oral Pathol* 1974;3:7-15.
31. Fowler BO, Kurdo S. Changes in heated and in laser-irradiated human tooth enamel and their probable effects on solubility. *Calcified Tissue International* 1986;38:197-208.
32. Hashiguchi K, Hashimoto K. Effect of Nd:Yag laser irradiation on human dental enamel. *Okajimas-Folea-Anat-Jpn* 1990; 67(4);271-80.
33. Tagomori S, Morioko T. Combined effects of laser and fluoride on acid resistance of human dental enamel. *Caries Res* 1989; 23:225-31.
34. Fox JL, Yu D, Otsuka M, Higuchi WI, Wong J, Powell GL. Initial dissolution rate studies on dental enamel after CO2 laser irradiation. *J Dent Res* 1992;71(7):1389-98.



35. Borggreven JM, Van Dijk JW, Driessens FC. Effect of laser irradiation on the permeability of bovine dental enamel. *Arch Oral Biol* 1980;25:831-2.
36. Ferreira JM, Palamara J, Phakey PP, Rachinger WA, Orams HJ. Effects of continuous wave CO<sub>2</sub> laser on the ultrastructure of human dental enamel. *Arch Oral Biol* 1989;34(7):551-62.
37. Stern RH, Sognnaes RF. Laser inhibition of dental caries suggested by first tests in vivo. *J Am Dent Assoc* 1972; 85:1087-90.
38. Hess JA. Scanning electron microscopic study of laser-induced morphologic changes of a coated enamel surface. *Lasers in Surgery and Medicine* 1990;10:458-62.
39. Marquez F, Quintana E, Roca I, Salgado J. Physical-mechanical effects of Nd:YAG laser on the surface of sound dental enamel. *Biomaterials* 1993;14(4):313-6.
40. Brunelle JA, Carlos JP. Changes in the prevalence of dental caries in U.S. school children. *J Dent Res* 1982;61:1346-51.
41. Sheiham A. Future patterns of dental care-manpower implications for industrialized countries. *Brit Dent J* 1989;166:240-2.
42. Featherstone JDB, Nelson DGA. Recent uses of electron microscopy in the study of physio-chemical processes affecting the reactivity of synthetic and biological apatites. *Scanning Microsc* 1989;3:815-28.
43. Quintana E, Marquez F, Roca I, Torres V, Salgado J. Some morphologic changes induced by Nd:YAG laser on the noncoated enamel surface: A scanning electron microscopy study. *Lasers Surg Med* 1992;12:131-6.
44. Walsh LJ, Perham SJ. Enamel fusion using a carbon dioxide laser: A technique for sealing pits and fissures. *Clin Prev Dent* 1991;13(3):16-20.



45. Stewart L, Powell GL, Wright S. Hydroxyapatite attached by laser: A potential sealant for pits and fissures. *Oper Dent* 1985;10(1):2-5.
46. Meurman JH, Voegel JC, Rauhamaa-Makinen R, et al. Effects of Carbon Dioxide, Nd:YAG and Carbon Dioxide-Nd:YAG combination lasers at high energy densities on synthetic hydroxyapatite. *Caries Res* 1992;26:77-83.
47. Arcoria CJ, Lippas MG, Vitasek BA. Enamel surface roughness analysis after laser ablation and acid etching. *J Oral Rehabil* 1993;20:213-24.
48. von Fraunhofer JA, Allen DJ. Thermal effects associated with the Nd/YAG dental laser. *Angle Orthod* 1993;63(4):299-303.
49. Adrian JC, Bernier JL, Sprague WG. Laser and the dental pulp. *J Am Dent Assoc* 1971;83:113-7.
50. Stern RH, Renger HL, Howell FV. Laser effects on vital dental pulps. *Br Dent J* 1969;127:26-8.
51. Leighty SM, Pogrel MA, Goodies HE, Marshall GW, White JM. Effects of the carbon dioxide laser on teeth [Abstract]. *J Dent Res* 1989;68:876.
52. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. *J Biol Chem* 1925;66:375-400.
53. Stern RH, Vahl J, Sognnaes RF. Lased enamel: Ultrastructural observations of pulsed dioxide laser effects. *J Dent Res* 1972;51:455-60.
54. McDonald RE, Avery DR, eds. *Dentistry for the child and adolescent*. 6th ed. St Louis: CV Mosby, 1994:60.
55. Morioka T, Suzuki K, Tagomori S. Effect of beam absorptive mediators on acid resistance of surface enamel by Nd:YAG laser irradiation. *J Dent Health* 1984;34:40-4.

## APPENDIXES



## APPENDIX I

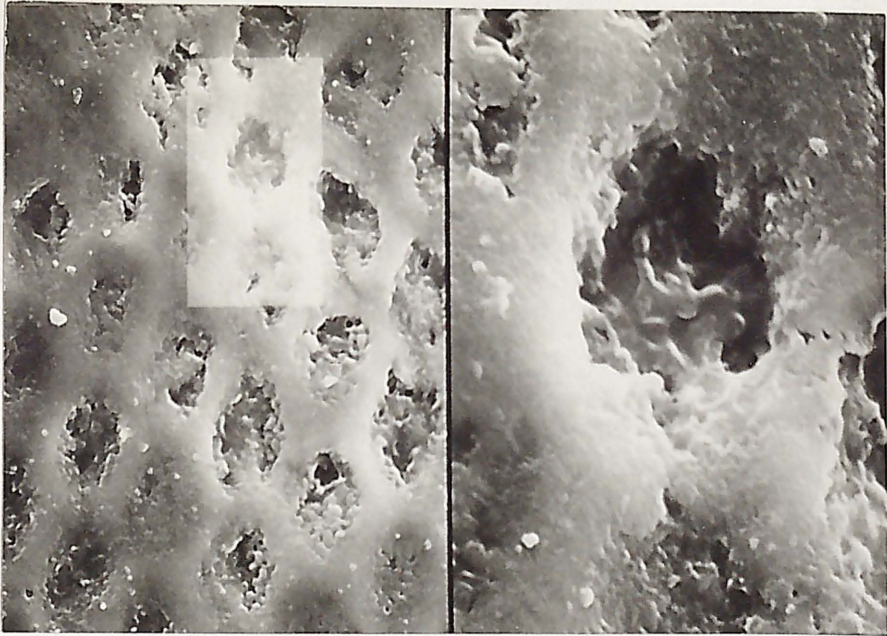


FIGURE 1. SEM: Permanent tooth #1. Pit and fissure surface. Control.

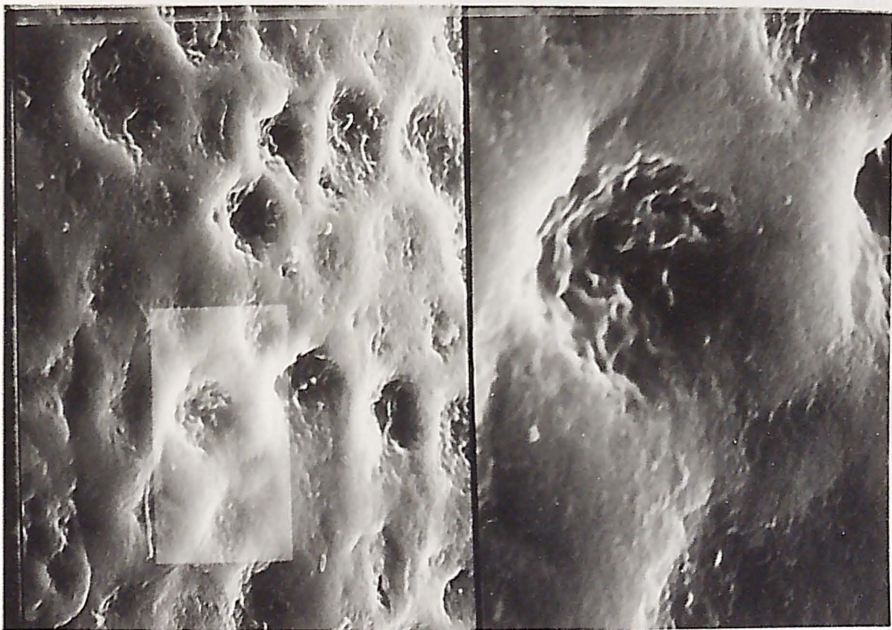


FIGURE 2. SEM: Permanent tooth #1. Pit and fissure surface. Lased at 5 watts.



## APPENDIX II

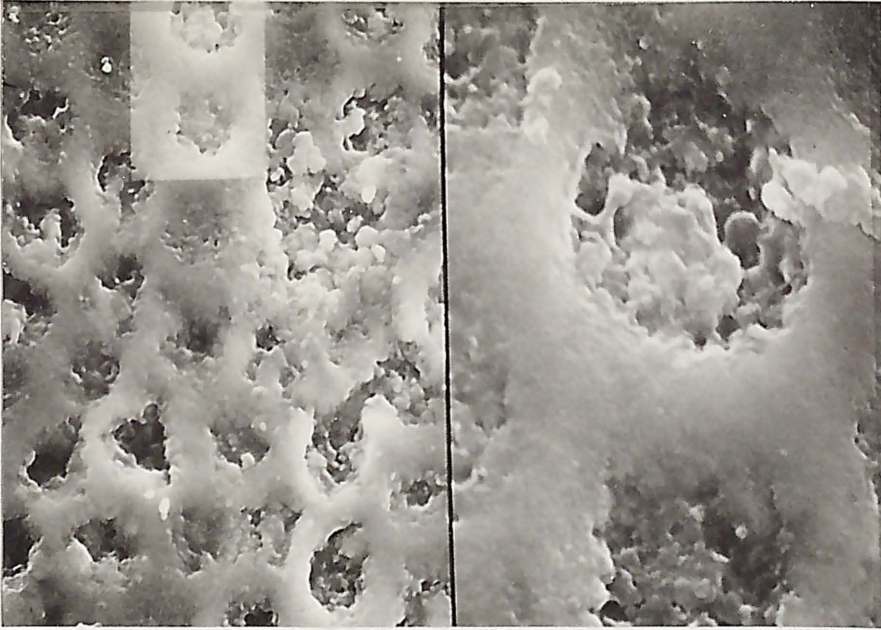


FIGURE 3. SEM: Permanent tooth #1. Pit and fissure surface. Lased at 10 watts.



FIGURE 4. SEM: Permanent tooth #1. Pit and fissure surface. Lased at 15 watts.



## APPENDIX III

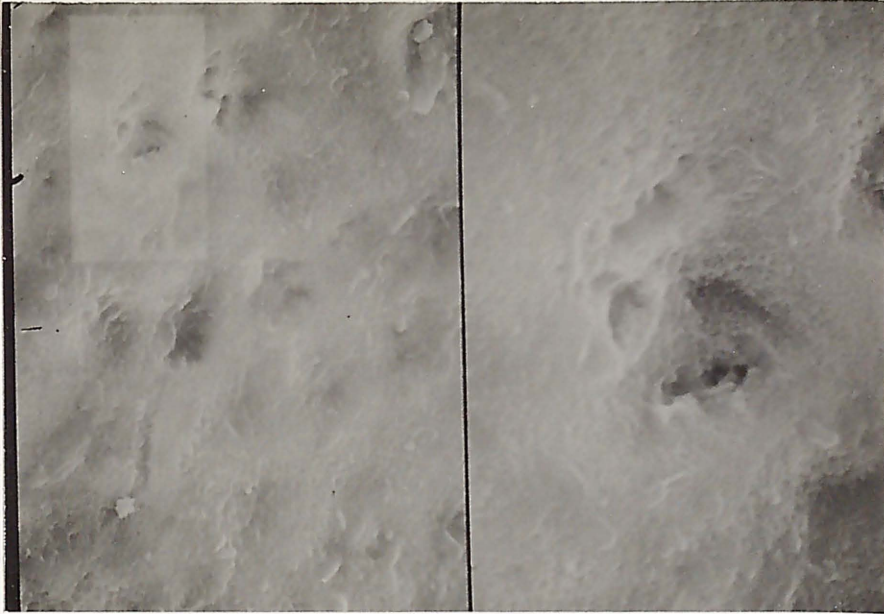


FIGURE 5. SEM: Primary tooth #1. Pit and fissure surface. Lased at 20 watts.

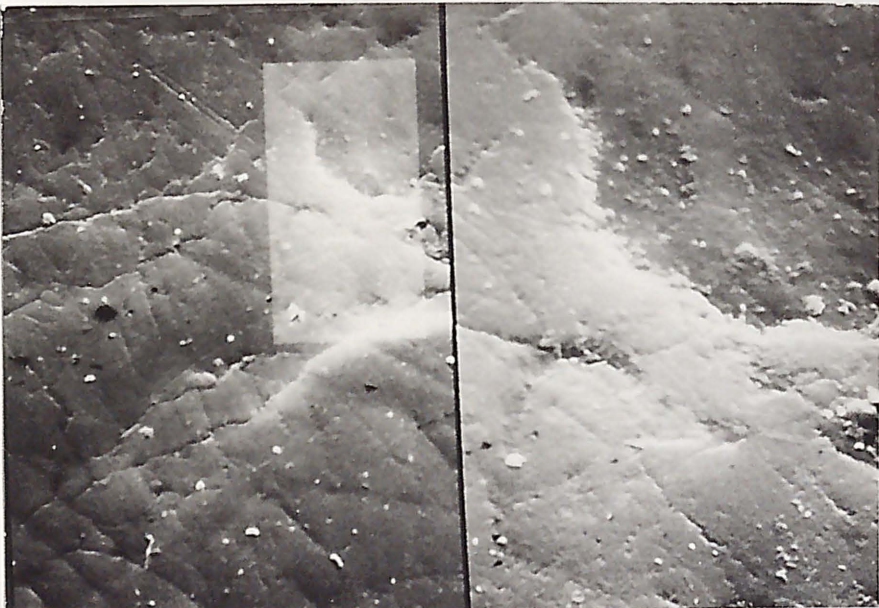


FIGURE 6. SEM: Primary tooth #1. Pit and fissure surface. Lased at 25 watts.

## APPENDIX IV

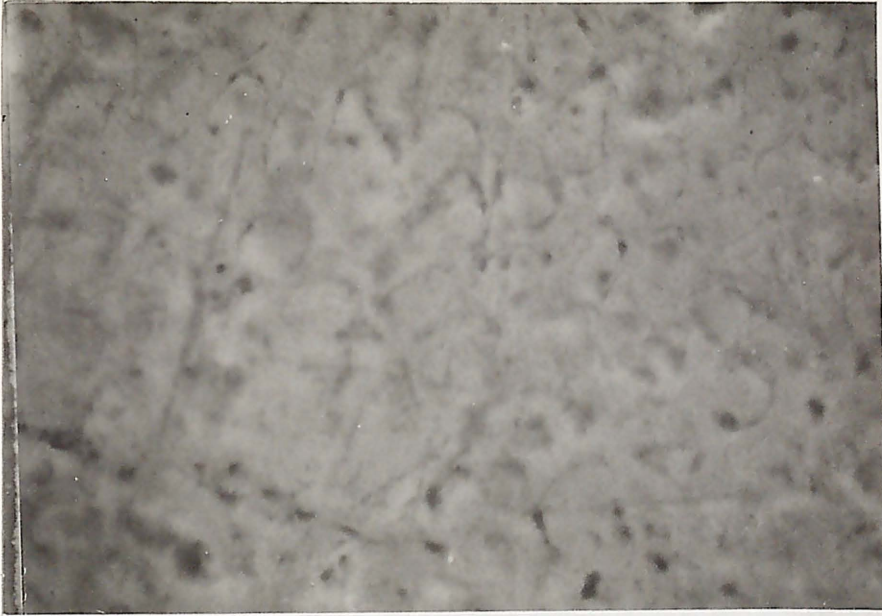


FIGURE 7. SEM: Permanent tooth #2. Pit and fissure surface. Pre laser treatment.

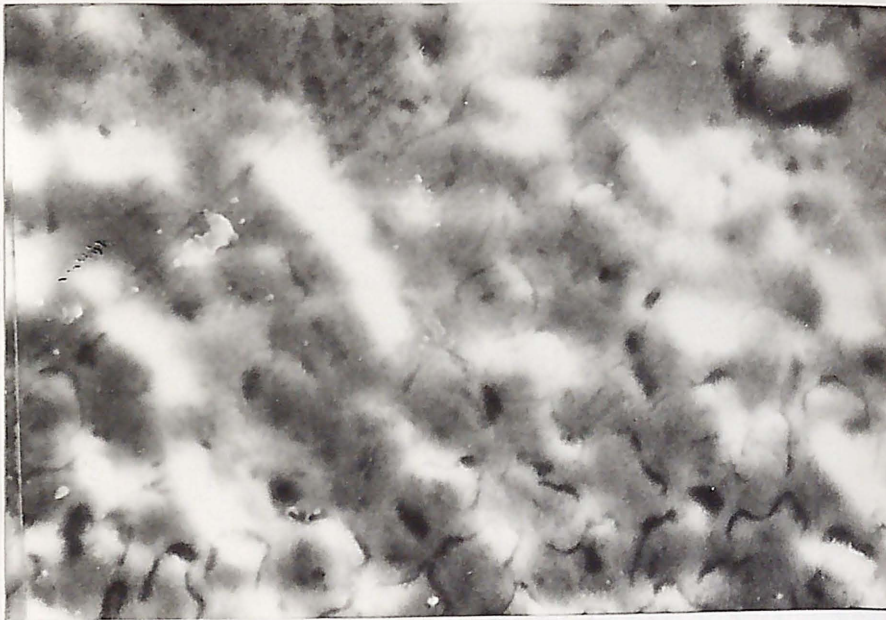


FIGURE 8. SEM: Permanent tooth #2. Pit and fissure surface. Post laser treatment at 20 watts in same area as above picture. Area shows more roughness.



## APPENDIX V

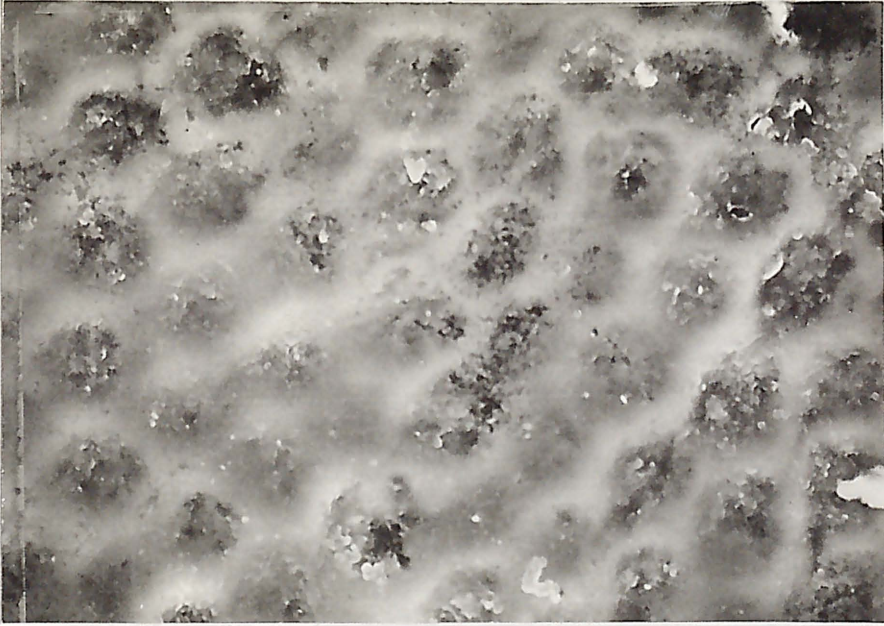


FIGURE 9. SEM: Permanent tooth #3. Smooth surface. Control. Enamel prisms indicated.

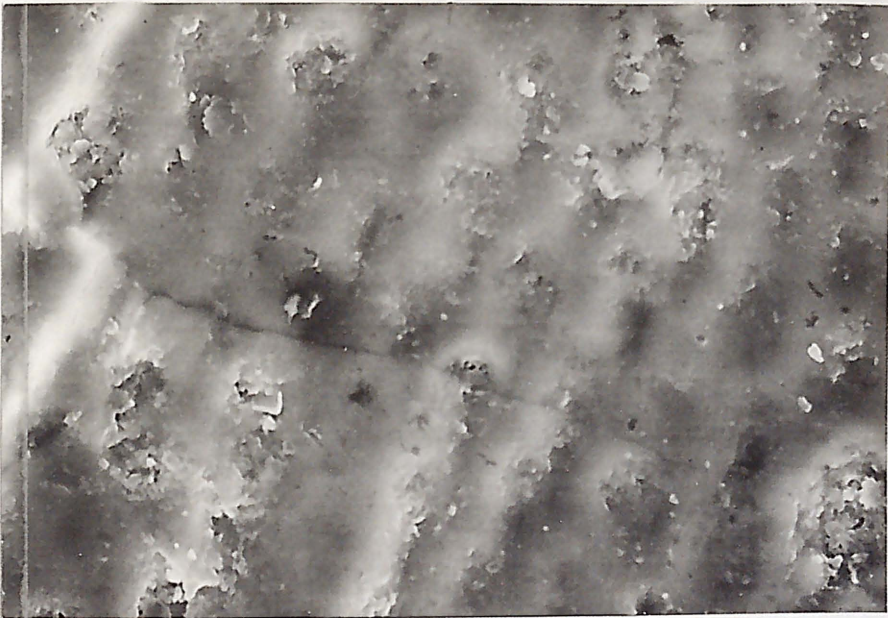


FIGURE 10. SEM: Permanent tooth #3. Smooth surface. Lased at 15 watts. Enamel appears smoother.



## APPENDIX VI

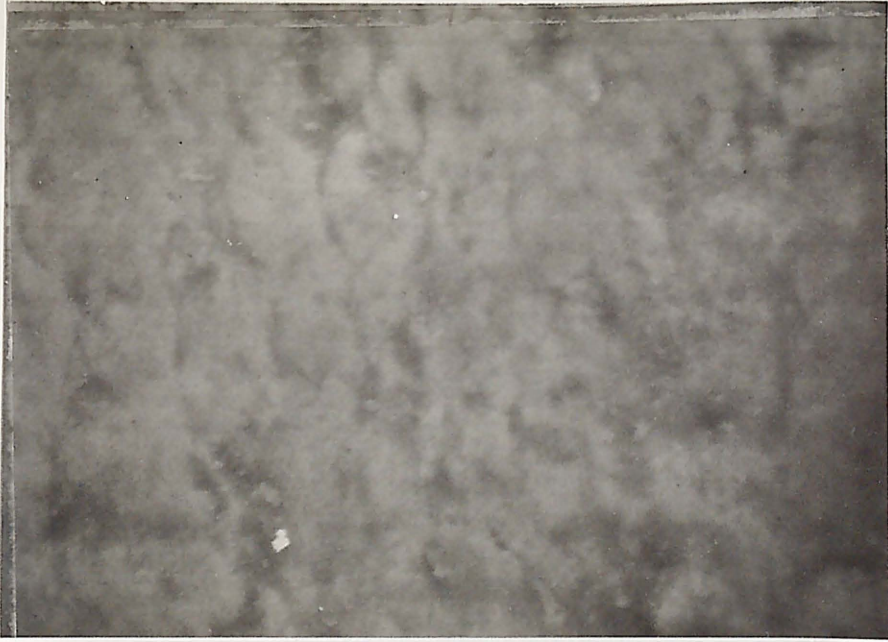


FIGURE 11. SEM: Permanent tooth #4. Pit and fissure. Control.

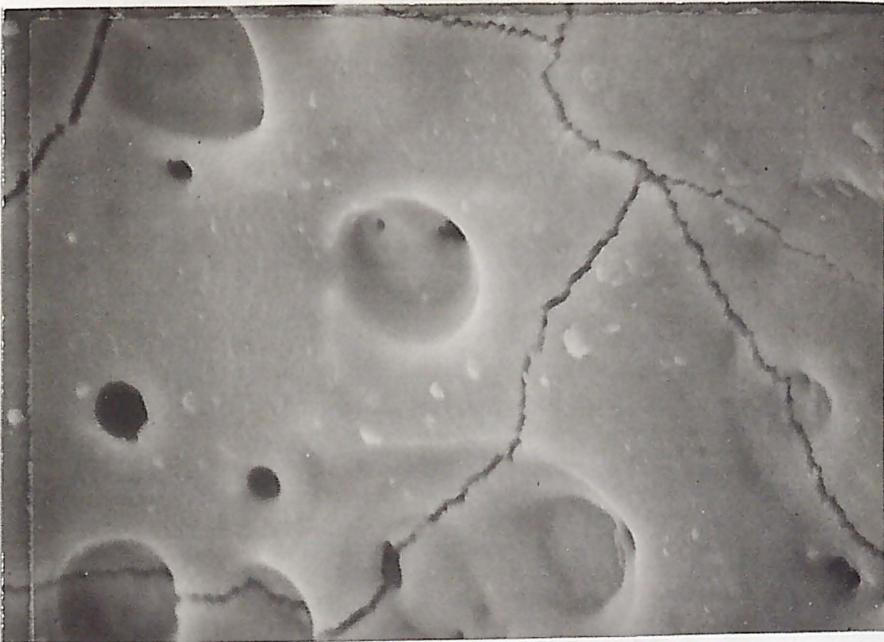


FIGURE 12. SEM: Permanent tooth #4. Pit and fissure. Lased at 20 watts. Craters and cracking and smoother surface is noted.



## APPENDIX VII

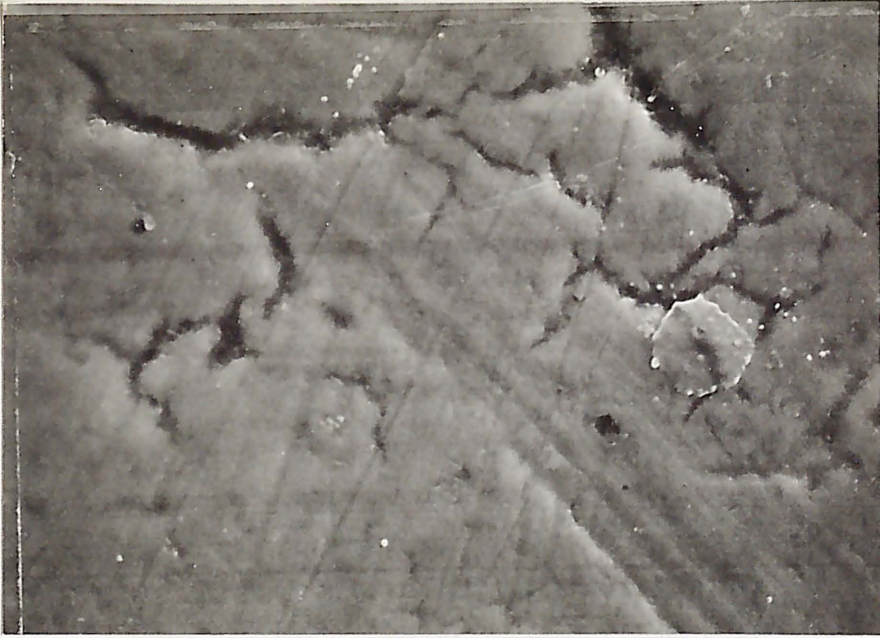


FIGURE 13. SEM: Permanent tooth #5. Smooth surface. Control.

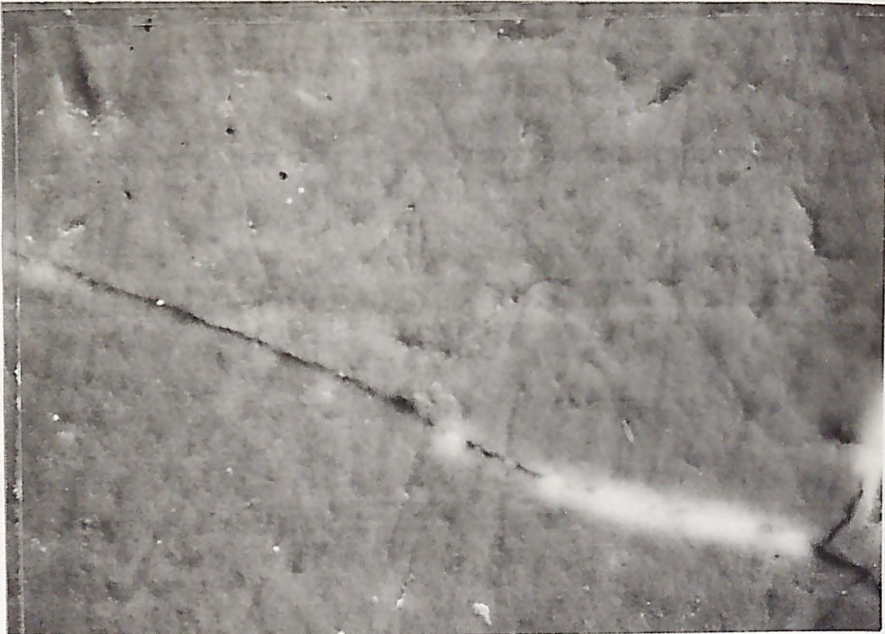


FIGURE 14. SEM: Permanent tooth #5. Smooth surface. Lased at 20 watts. Surface is smoother.



## APPENDIX VIII

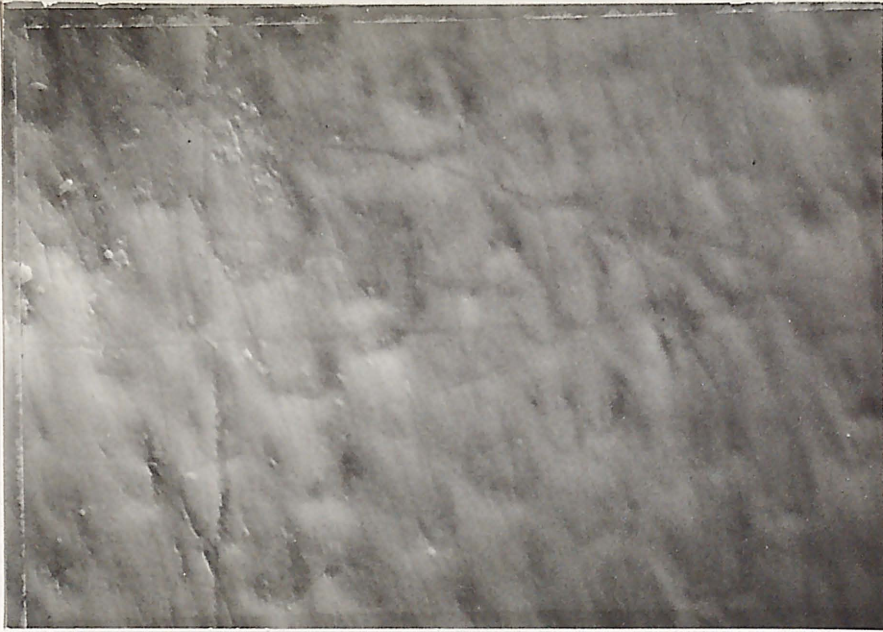


FIGURE 15. SEM: Permanent tooth #6. Pit and fissure. Control. Enamel prisms noted.

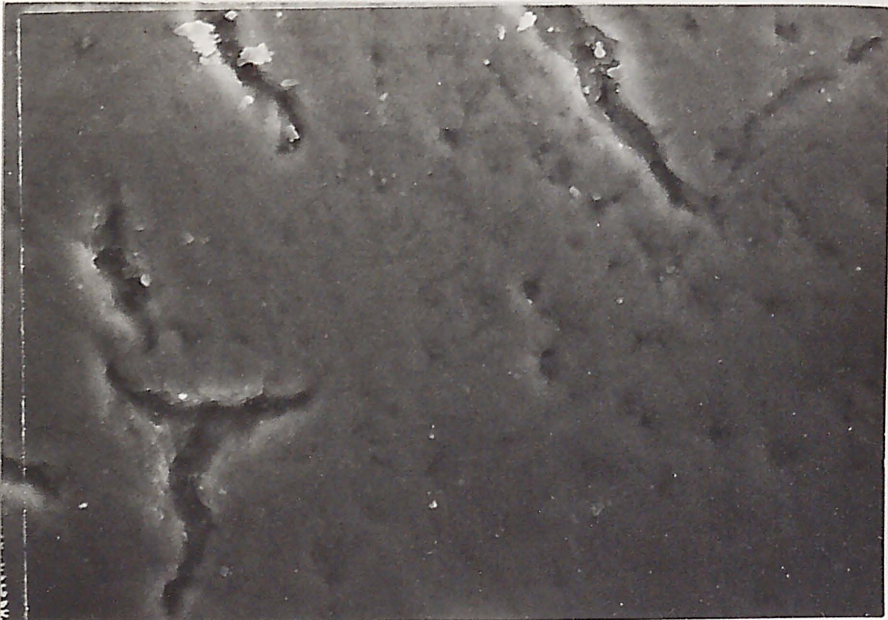


FIGURE 16. SEM: Permanent tooth #6. Pit and fissure lased at 25 watts. Smoother surface is seen.



## APPENDIX IX



FIGURE 17. SEM: Primary tooth #2. Lased at 20 watts. Cracking and pitting noted.



# APPENDIX X

All surface characteristics seen on smooth surface in all four areas.

Chip #	Area	Smooth	Cracks	Enamel Prisms	Cobblestone Bubbles	Pits	Rough	Craters	Particles
Per 1	L, AE	x							
	L, NAE	x							
	NL, NAE	x							
	NL, AE	x							
Per 2	L, AE	x							
	L, NE				x	slightly			
	NL, NE	x							
	NL, AE					slightly			
Per 3	L, AE	x							
	L, NE	x							
	NL, NE	x							
	NL, AE	x							
Per 4	L, AE	x							
	L, NE	x							
	NL, NE				x				
	NL, AE	x							
Pri 1	L, AE	x							
	L, NE	x							
	NL, NE	x							
	NL, AE	x							
Pri 2	L, AE				x		x		
	L, NE	x							
	NL, NE	x							
	NL, AE	x							
Pri 3	L, AE	x							
	L, NE	x				x			
	NL, NE	x				x			
	NL, AE			x					
Pri 4	L, AE		x				very		
	L, NE						very		
	NL, NE						very		
	NL, AE			x		x		x	large



# APPENDIX XI

All surface characteristics seen on pit and fissure surfaces in all four areas.

Chip #	Area	Pitting	Cratering	Cracking	Enamel Prisms	White Bubbles	Smooth	Rough	Grainy	Cobblestone	Ridges	Ravene
Per 1	L, AE	x	x	fine		x						
	L, NE		not as deep, shallow									
	NL, NE		deep									
	NL, AE		deep	x from craters								
Per 2	L, AE	x deep	x									
	L, NE	x not as deep						rougher	x			
	NL, NE	x not as deep	x						not as grainy			
	NL, AE				x			x				
Per 3	L, AE	severe, deeper	x				x					
	L, NE	x	x				x					
	NL, NE	shallow, few					x					
	NL, AE		shallow		x		x					
Per 4	L, AE					x	x			x		
	L, NE	x	x deep			x	x			x		
	NL, NE							x				
	NL, AE							x				
Pri 1	L, AE		x very few, shallow				x					
	L, NE						x				x	
	NL, NE						x					
	NL, AE	x few						x				
Pri 2	L, AE							x				
	L, NE	x some						x				
	NL, NE							x				
	NL, AE	shallow						x			x	
Pri 3	L, AE						x					
	L, NE	minor					x					x
	NL, NE	x					x					
	NL, AE							x				
Pri 4	L, AE				x			x				
	L, NE						x					
	NL, NE						x					
	NL, AE							x				

ABSTRACT



COMPARISON OF NEODYMIUM:YTTERIUM, ALUMINUM, GARNET  
LASER EFFECTS BETWEEN PRIMARY AND PERMANENT  
ENAMEL OF DISASSOCIATED TEETH

By

Nora Najeeb Tleel

Indiana University School of Dentistry  
Indianapolis, Indiana

While advances in lasers for soft tissue applications have drastically increased its usage in dentistry, laser use for hard tissue still needs development. This study was conducted to determine the difference between permanent and primary enamel after their exposure to the Nd:YAG laser. Four parameters were studied: Surface topography, acid resistance, surface hardness and caries-like lesion depth creation.

Differences between lased primary and permanent teeth were seen in two parameters. Calcium disassociation during acid attack was significantly higher for lased primary enamel ( $p=0.0126$ ), and lased primary enamel became softer while permanent enamel became harder ( $p=0.0001$ ).

While this study adds to the body of knowledge related to hard tissue laser use, further evaluation and research is needed prior to the routine use of the laser on primary enamel.

## CURRICULUM VITAE



## Nora Najeeb Tleel

September 21, 1967	Born to Najeeb and Lina Amman, Jordan
May 27, 1989	B.S., Biology Marquette University Milwaukee, Wisconsin
May 17, 1992	Doctor of Dental Surgery Marquette University School of Dentistry Milwaukee, Wisconsin
June 30, 1994	Pediatric Dentistry Certification James Whitcomb Riley Hospital for Children/ Indiana University School of Dentistry Indianapolis, Indiana
January, 1995	Master of Science in Dentistry. Indiana University School of Dentistry Indianapolis, Indiana

## Professional Organizations

American Dental Association  
American Academy of Pediatric Dentistry  
The Jordanian Dental Association  
Omicron Kappa Upsilon